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REVIEW

Role of ETS Family Transcription Factors in Vascular Development and Angiogenesis

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ABSTRACT. **The ETS family of transcription factors is defined by a conserved DNA-binding ETS domain that forms a winged helix-turn-helix structural motif. This family of transcription factors is involved in a diverse array of biological functions including cellular growth and differentiation, as well as organ development. Among the members of this family, ETS-1, ERG, Fli-1, TEL, and NERF-2 are expressed in endothelial cells and their progenitors. This review will summarize the role of ETS family transcription factors in vascular development and angiogenesis.**

Key words: vasculogenesis/angiogenesis/transcription factor/ETS

The vascular endothelium is a continuous monolayer of endothelial cells (ECs) lining the luminal surface of blood vessels. ECs are normally quiescent, but they have the ability to proliferate and form neo-vessels. Angiogenesis is the formation of neo-vessels from pre-existing blood vessels. It occurs in a wide range of physiological and pathological states, including embryogenesis, wound healing, diabetic retinopathy, rheumatoid arthritis, and growth of solid tumors.

Organ formation requires precise spatial and temporal coordination in the expression of multiple genes in individual cells. Transcription factors determine gene expression by binding to the specific DNA sequences within the promoter/enhancer regions of genes and allowing transcriptional activation or repression of genes. Therefore, transcription factors play fundamental roles in gene activity during vascular development and angiogenesis.

The processes of vascular development and angiogenesis

The vascular system is the first functional organ that develops in the embryo. The extraembryonic mesodermal cells, so-called hemangioblasts, aggregate and form blood islands in the yolk sac, where they differentiate into an external layer of endothelial cells (ECs) and an inner core of blood cells. These outer ECs constitute the primary vascular plexus. Similarly, the intraembryonic mesodermal hemangioblasts and/or angioblasts located in the proximal lateral mesoderm differentiate into ECs and organize the dorsal aorta. These processes are called vasculogenesis. Subsequently, neo-vessels are generated from the primary vascular plexus and become distributed throughout the entire body. This process is called angiogenesis. In the final process of vascular development, mesenchymal cells differentiate into mural cells (smooth muscle cells or pericytes), surround blood vessels, and make the vessels mature and stable (for a review, see Risau, 1997).

Blood vessels in the adult are composed of ECs and mural cells, and are already stabilized. Angiogenesis in the adult includes at least six sequential steps: i. detachment of pre-existing pericytes or vascular destabilization; ii. extracellular matrix degradation by endothelial proteases; iii. migration of ECs; iv. proliferation of ECs; v. tube formation by Ecs; and vi. reattachment of pericytes or vascular stabilization (for a review, see Hanahan, 1997). Additionally, the presence of endothelial progenitor cells (EPCs) has been identified in bone marrow. EPCs migrate to the site of angiogenesis via the bloodstream, differentiate into ECs, and support angiogenesis as vasculogenesis-type neovascularization (Asahara *et al*., 1997; Asahara *et al*., 1999).

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Expression of ETS family transcription factors during vascular development and angiogenesis

A variety of transcription factors including ETS family transcription factors are expressed in cells of endothelial lineage during embryonic vascular development and in ECs during angiogenesis in the adult. The ETS family of transcription factors is defined by a conserved DNA-binding ETS domain that forms a winged helix-turn-helix structural motif that binds to a purine-rich consensus sequence GGA(A/T) in the promoter region of target genes. In addition to DNA-protein interactions, protein-protein interactions with partner proteins play significant roles in the targeting of ETS domain proteins to specific promoters. The family is divided into several subclasses according to the position of the ETS domain and the presence of specific subdomains. It is now evident that ETS family transcription factors are involved in a diverse array of biological functions including cellular growth and differentiation, as well as organ development. Among the members of this family, ETS-1, ERG, Fli-1, TEL, and NERF-2 are thought to be involved in vascular development and angiogenesis (Fig. 1).

(1) ETS-1 (E26 transformation specific-1)

ETS-1 was first identified as the cellular progenitor of the viral oncogene *v-ets* in the genome of the avian leukemia retrovirus E26, and is the prototype of the ETS family transcription factors (Leprince *et al*., 1983; Nunn *et al*., 1983). ETS-1 is the first member of the ETS family, which is shown to be expressed in mesoderm lineage cells including ECs during embryogenesis (Vandenbunder *et al*., 1989). Moreover, increased expression of ETS-1 is observed in ECs of neo-vessels during tumor angiogenesis in the adult (Wernert *et al*., 1992). Maroulakou *et al*. (1994) compared the patterns of expression of ETS-1 and its closely related ETS-2 during murine embryogenesis, and found that ETS-1 was preferentially expressed in developing vascular structures, including the heart, arteries, capillaries, and meninges. In relation to the relatively specific expression pattern of ETS-1 in ECs in embryo, an EC-specific element for expression of ETS-1 has been identified in the first intron of the *ETS-1* gene (Jorcyk *et al*., 1997).

We have shown that ETS-1 is transiently expressed in ECs during angiogenesis or re-endothelialization after denudation (Iwasaka *et al*., 1996; Tanaka *et al*., 1998). VEGF and bFGF were potent inducers of ETS-1 in ECs (Iwasaka *et al*., 1996; Tanaka *et al*., 1999), and that this induction of ETS-1 in ECs was mediated by the activation of a classical MAP kinase, ERK1/2 (Tanaka *et al*., 1999; Sato *et al*., 2000). Our results further suggested that ETS-1 promoted angiogenesis (Iwasaka *et al*., 1996; Chen *et al*., 1997; Oda *et al*., 1999). Although no vascular abnormality was described in ETS-1 (–/–) mice (Bories *et al*., 1995; Muth-

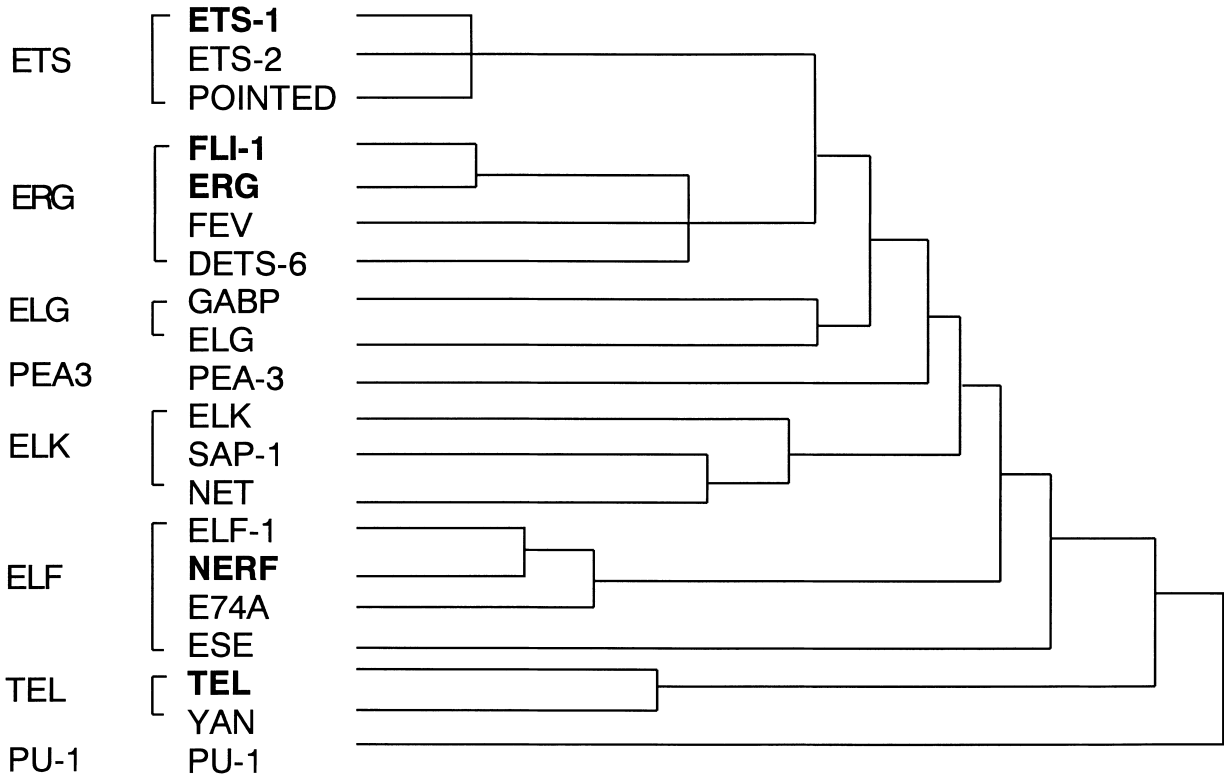

Fig. 1. The ETS family of transcription factors is subdivided into 8 subgroups. ETS-1, FLI-1, ERG, NERF, and TEL (bold) are expressed in ECs and their progenitors.

usamy *et al*., 1995), the elimination of the effect of ETS-1 by a dominant negative molecule inhibited angiogenesis in adult mice (Nakano *et al*., 2000). Therefore we assume that a molecule closely related to ETS-1 compensates for the missing ETS-1 in ETS-1 (–/–) mouse embryos to allow noraml vascular development.

(2) Fli-1 (Friend leukemia integration-site 1) and ERG (ETS-related gene)

Shortly after the evaluation of ETS-1 in the vascular system, other members of the ETS family were found to be expressed in ECs and their progenitors. Fli-1 is closely related to erythroleukemia, whose locus is disrupted in Friend murine leukemia virus (Ben-David *et al*., 1990). Fli-1 was found to be expressed in hemangioblasts, angioblasts, and ECs (Meyer *et al*., 1995; Melet *et al*., 1996). More recently, Brown *et al*. (2000) examined the expression pattern of Zebrafish Fli-1. Its expression was initially in the posterior lateral mesoderm, overlaping with that of GATA2, a marker of hematopoiesis, in a potential hemangioblast population. Subsequently, Fli-1 and GATA2 expression patterns diverged, with separate Fli-1 and GATA2 expression domains arising in the developing vasculature and sites of blood formation, respectively. Therefore, Fli-1 is one of the earliest indicators of hemangioblast formation. The function of Fli-1 was further examined by gene targeting in mice. Fli-1 (–/–) murine embryos were able to form a functional network of blood vessels, indicating that vasculogenesis and angiogenesis could proceed without this transcription factor. However, Fli-1 (–/–) murine embryos died at embryonic day 11.5 to 12.5, with a loss of vascular integrity leading to cerebral hemorrhage (Hart *et al*., 2000; Spyropoulos *et al*., 2000).

ERG is closely related to Fli-1, and is encoded on chromosome 21 (Rao *et al*., 1987). Vlaeminck-Guillem *et al*. (2000) recently reported that the *ERG* gene was expressed predominantly in mesodermal tissues, including the endothelial, precartilaginous and urogenital areas, as well as in migrating neural crest cells. When the Xenopus homologue of ERG was ectopically expressed in the ventral region of Xenopus embryos, ectopic endothelial differentiation was observed (Baltzinger *et al*., 1999). Therefore, ERG is suggested to play a role in endothelial differentiation. The expressions of ERG and Fli-1 in the mouse embryo were found to partially overlap (Vlaeminck-Guillem *et al*., 2000). However, the vascular defect in Fli-1 (–/–) murine embryos revealed that ERG was not able to compensate the function of Fli-1, at least in the vascular system.

(3) TEL (translocated ETS leukemia)

TEL is a sequence-specific transcriptional repressor of ETS-driven transcription (Golub *et al*., 1994; Poirel *et al*., 1997; Lopez *et al*., 1999). TEL binds to Fli-1 and inhibits its transcriptional activity (Kwiatkowski *et al*., 1998). TEL is widely expressed in various cells including ECs throughout embryonic development and in the adult (Wang *et al*, 1997; Lopez *et al*., 1999). TEL (–/–) murine embryos exhibited normal vasculogenesis but defective yolk sac angiogenesis, and intra-embryonic apoptosis of mesenchymal and neural cells (Wang *et al*., 1997). Thus, TEL is thought to be required for maintenance of the vascular network in the yolk sac and for survival of selected cell types within the embryo proper. More recently, Edel (1999) examined the expression of TEL during angiogenesis in the adult. It was evident that, although mature vessels expressed TEL, neo-vessels in either tumor or ovarian angiogenesis did not express TEL.

(4) NERF2 (new ETS-related factor 2)

NERF is a transcription factor closely related to the ETS family member ELF-1 (Oettgen *et al*., 1996). Three alternative splice forms of NERF, i.e., NERF1a, NERF1b, and NERF2, are produced from a single *NERF* gene. Among them, only NERF2 functions as a transcriptional activator, and is preferentially expressed in ECs (Dube *et al*., 1999; Iljin *et al*., 1999).

Molecules involved in angiogenesis

A variety of factors have been identified as regulators of vasculogenesis and angiogenesis. Among them, vascular endothelial growth factor (VEGF) and angiopoietins play principal roles. VEGF binds to endothelium-specific receptor-type tyrosine kinases, Flt-1 (VEGFR-1) and KDR/Flk-1 (VEGFR-2), and to the membrane protein neuropilin-1, which does not contain a tyrosine kinase domain. Among them, the VEGF/VEGFR-2 mediated signaling is indispensable for the differentiation of both ECs and blood cells from common precursor hemangioblasts, as occurs in vasculogenesis and primitive hematopoiesis (Shalaby *et al*., 1995; Ziegler *et al*., 1999). Although Flt-1 does not play a positive role in embryonic vascular development (Fong *et al*., 1995; Hiratsuka *et al*., 1998), we have reported that Flt-1 does play a role in the migration of differentiated ECs via the activation of p38 MAP kinase (Kanno *et al*., 2000).

Four angiopoietins, namely, angiopoietin-1 (ang-1), angiopoietin-2 (ang-2), angiopoietin-3 (ang-3), and angiopoietin-4 (ang-4), have been identified. Ang-1 and ang-4 are agonistic ligands of TIE-2, another endothelium-specific receptor-type tyrosine kinase. They induce tyrosine-phosphorylation of TIE2. In contrast, ang-2 and ang-3 are antagonistic ligands. They bind to TIE-2, but do not induce tyrosinephosphorylation, and thus shut down the TIE-2 mediated signaling. Angiopoietin/TIE-2 mediated signaling is indispensable for angiogenesis, recruitment of mural cells, as well as definitive hematopoiesis (Sato *et al*., 1995; Suri *et al*., 1996; Takakura *et al*., 1998). TIE-1 is closely related to TIE-2, although the ligand for TIE-1 is obscure at the moment. TIE-1 (–/–) murine embryos developed a normal vasculature during embryonic development, but exhibited impaired vascular integrity as evidenced by edema and localized hemorrhage (Sato *et al*., 1995).

The coordinated induction of proteases and cell migration is a principal feature of ECs in the initial step of angiogene-

sis. Matrix metalloproteinases (MMPs) and plasminogen activators (PAs) are required for the degradation of basement membranes, and integrins are required for cell migration. When ECs are stimulated with angiogenic factors such as VEGF, they express PAs, MMPs, and integrins (Senger, 1996).

Vascular endothelial (VE)-cadherin is a calcium-dependent endothelium-specific adhesion molecule, and is responsible for tube formation by ECs (Vittet *et al*., 1997).

Possible target molecules of ETS family transcription factors for angiogenesis

For a better understanding of the function of ETS family transcription factors at the molecular level, target molecules of ETS family transcription factors in ECs should be identified.

It is evident that a number of angiogenesis–related molecules such as MMPs and urokinase-type PA (u-PA) contain ETS-binding motifs in their promoter/enhancer regions. We demonstrated that the specific elimination of ETS-1 expression by antisense oligodeoxynucleotides inhibited the induction of u-PA and MMP-1 in ECs (Iwasaka et al., 1996). Thereafter, we established both high and low ETS-1-expressing EC lines by transfecting a EC line with *ETS-1* cDNA in a sense or antisense orientation (Oda *et al*., 1999). A high ETS-1-expressing EC line exhibited increased expression of MMP-1, MMP-3, and MMP-9, as well as the integrin β3 subunit; whereas a low ETS-1-expressing EC line exhibited decreased expression of those genes in comparison with their expression in the parental cell line. These results suggest that MMP-1, MMP-3, MMP-9, u-PA, and integrin β3 are target genes of ETS-1 in ECs.

The promoter of Flt-1 (VEGFR-1) contains multiple ETS binding sites, and the expression of Flt-1 is dependent on ETS family transcription factors (Wakiya *et al*., 1996). Among various ETS family transcription factors tested, ETS-1 and ETS-2 exhibited the strongest inducing activity on the Flt-1 promoter *in vitro* (Dube *et al*., 1999). We observed that VEGF induced the coordinated expression of ETS-1 and Flt-1 in ECs, whose expressions were mediated via VEGF/VEGFR-2 signaling (Sato *et al*., 2000). Indeed, a highly significant correlation was evident between ETS-1 and Flt-1 expression in ECs of the human glioma microvasculature *in vivo* (Valter *et al*., 1999).

ETS binding sites along with GATA and SCL/tal-1 sites were identified as critical elements for the expression of mouse Flk-1 (VEGFR-2) in transgenic mice (Kappel *et al*., 2000). Moreover, ETS-1 was coexpressed with Flk-1 during mouse embryonic development and tumor angiogenesis, and was shown to activate the Flk-1 promoter via ETS binding sites (Kappel *et al*., 2000).

The promoter of TIE-2 contains a cluster of ETS binding sites, and NERF-2 exhibited the strongest inducing activity on the TIE-2 promoter among the ETS family of transcription factors at least *in vitro* (Dube *et al*., 1999). However, Fli-1 (–/–) mice showed decreased expression of TIE-2 during embryonic development (Hart *et al*., 2000), a finding which may suggest that Fli-1 is an important regulator for the expression of TIE-2 in the mouse embryo.

The promoter of TIE-1 also contains a cluster of ETS binding sites (Iljin *et al*., 1999). Among members of the ETS family tested, NERF-2 and ETS-2 showed the strongest transactivation of the TIE-1 promoter *in vitro* (Iljin *et al*., 1999).

The promoter of VE-cadherin contains two ETS-binding sites along with a GT box, and ETS binding sites were found to be essential for VE-cadherin promoter activity (Gory *et al*., 1998). Overexpression of ETS-1 in cultured ECs increased the expression of VE-cadherin, whereas an ETS-1 antisense oligonucleotide and a dominant negative mutant of ETS-1 decreased its expression (Lelievre *et al*., 2000).

Table I summarizes the possible target molecules of ETS family transcription factors in ECs for the regulation of angiogenesis. Obviously, this family of transcription factors plays an important role in angiogenesis.

Conclusion

Angiogenesis is a complex phenomenon, that involves migration, proliferation, differentiation, and morphogenesis. A number of molecules are expressed in ECs during angiogenesis. Thus, transcriptional regulation of gene expression in ECs has become an important issue for understanding the molecular mechanisms of angiogenesis. Increasing evidence suggests that the ETS family of transcription factors plays an important role in angiogenesis. However, our understanding about them is still limited, since ETS binding motifs are found in the promoter/enhancer region of numerous genes. Further study is required to clarify the entire role of the ETS family of transcription factors in the biology of ECs.

Table I. POSSIBLE TARGET MOLECULES OF ETS FAMILY TRANSCRIPTION FACTORS FOR ANGIOGENESIS

Receptor tyrosine kinases	Flt-1 (VEGFR-1)
	KDR/Flk-1 (VEGFR-2)
	TIE-1
	TIE-2
Proteases	u-PA
	MMP-1
	MMP-3
	MMP-9
Adhesion molecules	Integrin β3
	VE-cadherin

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