Regulation of Vascular Inflammation and Remodeling by ETS Factors

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Abstract—The ETS (E26 Transformation-specific Sequence) factors are comprised of a family of transcription factors that share a highly conserved DNA binding domain. Although originally described for their role as protooncogenes in the development of several types of human cancer, they have subsequently been shown to regulate a wide variety of biological processes including cellular growth and differentiation under normal and pathological conditions. As transcription factors, they can either function as activators or repressors of gene expression. Several ETS family members are expressed in cells of vascular origin, including endothelial cells and vascular smooth muscle cells, where they regulate the expression of a number of vascular-specific genes. In the past few years, emerging evidence supports a novel role for selected ETS family members in the regulation of vascular inflammation and remodeling. ETS factor expression can be induced by proinflammatory cytokines, growth factors, and vasoactive peptides. Examples of some of the target genes regulated by ETS factors include adhesion molecules, chemokines, and matrix metalloproteinases. Targeted disruption of selected ETS family members such as Ets-1 in mice is associated with marked reductions in the recruitment of inflammatory cells and vascular remodeling in response to systemic administration of the vasoactive peptide angiotensin II. The purpose of this review is to provide an overview of recent advances that have been made in defining a role for selected members of the ETS transcription factor family in the regulation of vascular-specific gene expression, vascular inflammation, and remodeling. (Circ Res. 2006;99:1159-1166.)

Key Words: gene regulation ■ inflammation ■ remodeling ■ transcription factor ■ ETS factor

Several transcription factors have been shown to mediate vascular inflammatory responses. The prototypic transcription factor for mediating these responses is nuclear factor κB (NF-κB). The protein constituents of NF-κB are expressed in many cells but remain inactive within the cytoplasm through binding to the inhibitory protein IκB (inhibitor of NF-κB). In response to inflammatory stimuli, IκB is released and the active form of NF-κB translocates to the nucleus, where it binds to and activates the expression of a number of inflammatory genes.1 In addition to NF-κB, the so-called “immediate-early genes,” including c-jun and c-fos mediate early inflammatory responses.2,3

More recently selected transcription factors have been identified that exhibit antiinflammatory properties and can modulate the initial cascade of genes induced in response to inflammatory stimuli. For example, the peroxisome proliferators-activated receptor (PPAR) nuclear receptors are transcription factors expressed in endothelial cells (ECs), vascular smooth muscle cells (VSMCs), and monocytic cells. Activation of PPARα and PPARγ receptors are associated...
with favorable effects on lipid metabolism and insulin sensitivity that are also beneficial with regard to limiting the development of atherosclerosis.4 Binding of PPAR agonists to their cognate receptors is also associated with antiinflammatory effects. Activation of the PPARγ pathway, for example, can inhibit the activity of the transcription factors activator protein-1 and NF-κB in response to proinflammatory cytokines such as tumor necrosis factor (TNF)-α in ECs.5

Historically, transcription factors have not been viewed as good targets for drug therapy, with the exception of nuclear hormone receptors that often reside on the cell surface and are activated by ligands that promote their transfer into nucleus, where they function as transcription factors and bind to specific gene targets. The ability to identify small molecules that specifically block transcription factors that are not ligand dependent has recently been demonstrated.6 The elucidation of the critical transcriptional factors that regulate vascular inflammation, therefore, may not only advance our basic understanding of the molecular mechanisms of vascular inflammation but also provide novel therapeutic targets for drug discovery.

ETS Transcription Factor Family

The ETS factors are a family of transcription factors that share a highly conserved DNA-binding domain (Ets domain). The name “ETS” originates from a sequence that was detected in an avian erythroblastosis virus, E26, where it formed a transforming gene together with Δgag and c-myb.7,8 This newly discovered sequence was called E26 Transformation-specific Sequence, or ETS. A cellular homologue of the viral ETS was subsequently identified. There are approximately 25 to 30 ETS family members identified. These are highly conserved orthologs of the individual ETS factors exist in several different species including human, mouse, chicken, Xenopus, nematodes, and drosophila. ETS factors are involved in regulating a wide variety of biological processes including normal development and differentiation.9 As protooncogenes, they have also been implicated in the pathogenesis of several different types of cancer.10,11

The highly conserved Ets domain contains three α-helices and four stranded β-sheets forming a winged helix–turn–helix (wHTH) structure. Contact to the major groove of DNA is mediated by the third α-helix. Although all ETS factor bind to a core “GGAA/T” nucleotide sequence, further specificity in binding is defined by flanking DNA core motif. Alterations in single amino acids can lead to changes in the DNA binding specificity. In addition to the Ets domain, there are several other important structural domains. The Ets-1 protein, for example contains 2 α-helical inhibitory domains that flank the Ets domain (Figure 1). The inhibitory activity of these domains can be blocked through phosphorylation or through protein/protein interactions. Two additional domains include the transactivation domain (TAD) and the pointed (PNT) domain that also promote protein-protein interactions. The PNT domain, named after a similar domain in the Ets-1–related drosophila ETS factor Pointed, is also found in several other mammalian ETS factor orthologs and consists of 5 α helices.12 Examples of several other transcription factors that Ets-1 interacts with include acute myeloid leukemia-1, activator protein-1, GATA3, hypoxia-inducible factor-2α, c-Myb, NFAT (Nuclear Factor of Activated T lymphocytes), NF-κB, Sp1, and Stat5.13

In addition to a highly conserved DNA binding domain (Ets domain), the different ETS family members have shared as well as distinct structural domains (Figure 2). For example, the ETS factor ESE-1 has 2 DNA binding domains: a classical Ets domain and a unique A/T hook domain.14 Although most of the Ets family members function to upregulate gene expression others such as TEL and NET act as transcriptional repressors, several other ETS family members contain inhibitory domains similar to Ets-1.

The transcriptional activity of ETS factors can be further modulated through a number of posttranslational modifications.15 The activity of most ETS factors can be modulated through phosphorylation. The function of Ets-1, for example, can be positively and negatively regulated through phosphorylation. Calmodulin-dependent kinase II (CaMKII) inhibits
DNA binding through serine phosphorylation of Ets-1 inhibitory domains. In contrast, phosphorylation of threonine-38 by the mitogen-activated kinases extracellular signal-regulated kinases 1 and 2 potently increases the transcriptional activity of Ets-1.

Another mechanism by which the activity of transcription factors is regulated posttranslationally is through lysine modifications. Two forms of lysine modifications that are known to modify the function of selected ETS transcription factors include sumoylation and acetylation. Sumoylation involves the ligation of the Sumo protein to lysine residues via the E2 enzyme Ubc9. Sumoylation of Fli-1 results in repression of transcriptional activity of the ETS factor Fli-1.

Sequence analysis of the Ets-1 protein reveals four potential lysines (amino acid 15, 200, 227, and 435) in regions matching the $\psi$KXE/D sumoylation consensus sequence, where $\psi$ represents a hydrophobic amino acid and X refers to any amino acid. Sumoylation of Ets-1 in unstimulated fibroblasts was recently shown to predominantly occur at lysine 15.

Another important mechanism by which the function of ETS factors can be regulated is through nuclear transport. In order for the ETS factors to function as transcription factors they must be localized within the nucleus. Specific regions called nuclear localization sequences (NLSs) within each of the ETS family members facilitate their movement from the cytoplasm into the nucleus. Two NLSs exist within the Ets domain of Ets-1 protein. Deletion of either of these regions results in accumulation of the mutated Ets-1 protein within the cytoplasm. Nuclear import of transcription factors occurs through the formation of a nuclear pore complex, involving the interaction of soluble carrier proteins of the karyopherin/importin family with the nuclear localization sequences within the transcription factors. The nuclear localization of the ETS factor PU.1 requires the binding of the NLS of PU.1, located within the Ets domain, with the nucleoporin Nup153, the GTPase Ran, and GTP to form a PU.1-Ran-GTP-Nup153 complex. Formation of this complex facilitates the energy dependent transport of PU.1 into the nucleus, whereby Ran-GTP facilitates movement of PU.1 across the nuclear pore to the nucleoporin Nup153.

Regulation of Endothelial-Specific Genes by ETS Factors

Several studies support a role for ETS factors in the regulation of endothelial-specific gene expression. Ets-1 has been shown to regulate genes involved in endothelial function and angiogenesis. Ets-1 enhances endothelial migration by enhancing the expression of matrix metalloproteinases (MMPs) and $\beta_3$ integrin. Ets-1 also regulates the expression of other genes involved in angiogenesis including the vascular endothelial growth factor receptors and angiopoietin-2. Dominant-negative forms of Ets-1 exhibit antiangiogenic activity in cultured ECs. Whereas targeted disruption of Ets-1 is not associated with a particular defect in vascular development, inhibition of both Ets-1 and Ets-2 in the developing chicken leads to defective coronary vessel development.
Regulation of Acute Vascular Inflammation by ETS Factors

Until recently, very little was known about a role for ETS factors in regulating vascular inflammation. Over the past few years several studies have been completed that support a role for several ETS family members in the regulation of vascular inflammation, including endothelial activation in response to inflammatory mediators, the recruitment of inflammatory cells to the vessel wall, and proliferation and migration of VSMCs (Figure 4, Table 1). We and others have observed that Ets-1 is induced in VSMCs and ECs in response to a variety of stimuli including angiotensin II (Ang II), platelet-derived growth factor (PDGF)-BB, thrombin, interleukin (IL)-1β, and TNF-α. Target genes identified to be downstream of Ets-1 in the setting of acute vascular inflammation include the chemokine monocyte chemotactic protein (MCP)-1, and the adhesion molecule vascular cellular adhesion molecule (VCAM)-1. Systemic administration of the vasoactive peptide Ang II via continuous infusion is not only associated with increases in blood pressure but also promotes the recruitment of inflammatory cells, including T cells and monocyctic cells, to the vessel wall. The influx of inflammatory cells in response to Ang II is markedly diminished in Ets-1–deficient mice compared with littermate controls. One of the major mediators of vascular inflammation within the vessel wall is reactive oxygen species (ROS). Ang II, for example, promotes the generation of superoxide anions in VSMCs largely via the activity of NAD(P)H oxidases, that can be converted to hydrogen peroxide by superoxide dismutase. ROS, and in particular hydrogen peroxide, can also stimulate Ets-1 expression. Ets-1 functions synergistically with the transcription factor Sp1 to regulate the expression of the PDGF receptor in an ROS-dependent manner. Ets-1 and Sp1 are enriched in VSMCs found in human atherosclerotic lesions that express increased levels of the PDGF receptor.

The ETS factor ESE-1 was originally identified as an epithelial-specific ETS factor. Under noninflammatory conditions, this ETS factor is only expressed in cells of epithelial origin. However, in response to inflammatory stimuli such as endotoxin or proinflammatory cytokines, including IL-1β or TNF-α, this transcription factor is highly induced in cultured primary ECs or VSMCs. In a mouse model of endotoxemia, ESE-1 is rapidly induced in the endothelium and first medial layer of smooth muscle cells of the mouse aorta. Target genes regulated by ESE-1 include NO synthase 2 (NOS2) and cyclooxygenase (COX)-2. ESE-1 has also recently been shown to function in the regulation of TNF-α mediated expression of Angiopoietin-1. The transcriptional activity of ESE-1 can be positively and negatively modified by its interaction with other proteins. Whereas binding of ESE-1 to CBP and p300 is associated with an increase in the transcriptional activity of ESE-1, the interaction with the Ku proteins Ku70 or Ku86 represses ESE-1 function.

Heme oxygenase-1 (HO-1) is a cytoprotective enzyme that is rapidly induced in monocytic cells in response to inflammatory stimuli such as endotoxin. The ETS factor Elk-3 functions as a potent transcriptional repressor that binds to

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<td>MCP-1, VCAM-1, PAI-1, IL-2, IFN-γ, Sm22α, PDGFβ</td>
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<td>Elk-3</td>
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regulatory sites within the HO-1 promoter and thereby inhibits the transcriptional activity of this promoter.63 In response to endotoxin mRNA levels of Elk-3 rapidly diminish in cultured primary macrophages, associated with increased HO-1 levels. Under basal conditions, Elk-3 functions as a potent repressor of HO-1 expression, thereby contributing to transcriptional regulation of HO-1 gene under inflammatory and noninflammatory conditions. Elk-3 similarly functions as a repressor of NOS2 gene expression under noninflammatory conditions.64

**Regulation of Vascular Remodeling by ETS Factors**

Acute inflammatory responses in the vessel wall may be followed by VSMC hypertrophy and neointimal formation. Ets-1 expression is induced in the rat carotid artery after balloon injury and promotes the proliferation and migration of VSMCs.49,65 Other downstream targets of Ets-1 that are expressed by VSMCs and promote cell migration include the MMPs stromelysin (MMP-3) and type IV collagenase (MMP-2).66 Ets-1 expression in VSMCs also induces the expression of PDGF, thereby promoting VSMC proliferation.55

In addition to the proliferation of VSMCs, neointimal formation is associated with phenotypic modulation of VSMCs that results in a reduction in the expression of VSMC-specific marker genes including smooth muscle α actin, smooth muscle myosin heavy chain, and SM22α.67 Ets-1 expression in VSMCs promotes a dedifferentiated state that is associated with increased proliferation and decreased expression of VSMC-specific genes.68 The ETS factor Elk-1 can also function as a repressor of SM22α and telokin in VSMCs.69 A role for telomerase in the regulation of several critical cellular functions in VSMCs, including cell proliferation, differentiation, and the replicative lifespan of the cell has recently been demonstrated.70 Activation of the nuclear hormone receptor PPARγ inhibits telomerase activity and VSMC proliferation in response to PDGF-BB. The mechanism by which PPARγ reduces telomerase activity is via a reduction of the expression of telomerase reverse transcriptase (TERT) that regulates the catalytic activity of telomerase. The decrease in TERT expression is at least in part mediated through a reduction in Ets-1 expression.70 Chronic exposure of VSMCs to Ang II in blood vessels is associated with a hypertrophic response. VSMCs isolated from Ets-1−/− deficient mice exhibit decreased proliferative responses to Ang II, and systemic administration of Ang II to Ets-1−/− deficient mice is associated with marked reductions in medial hypertrophy, compared with littermate controls, despite similar increases in blood pressure.71

Significant reductions in perivascular fibrosis are also observed in Ets-1−/− deficient mice after infusion of Ang II compared with control animals.52 Plasminogen activator inhibitor (PAI)-1 has been shown to be critical for the development of perivascular fibrosis associated with several animal models of hypertension.72 Exposure of VSMCs and ECs to Ang II leads to rapid induction of PAI-1 expression in these cells. Whereas in plasma, PAI-1 acts as a critical determinant of the fibrinolytic system, in vascular tissue it acts to modulate inflammatory responses by inhibiting cellular migration and matrix degradation.73−75 The generation of plasmin, which is inhibited by PAI-1, can activate latent MMPs that are involved in remodeling of the extracellular matrix.76,77 Induction of PAI-1 in the setting of vascular inflammation leads to a reduction in MMP activity and increased collagen deposition, thereby promoting increased fibrosis.78 The induction of PAI-1 in VSMCs and ECs of the aorta in response to Ang II is significantly reduced in Ets-1−/− deficient mice compared with littermate controls, suggesting that a reduction in PAI-1 may at least in part explain the diminished perivascular fibrosis observed in Ets-1−/− mice treated with Ang II.52

Vascular remodeling can occur in several vascular diseases. Ets-1 is highly expressed in VSMCs derived from human carotid atherosclerotic plaques.79 Another downstream target of Ets-1 identified in these cells is the atherosclerotic lesions is Fas ligand. Ets-1 functions synergistically with the transcription factor Sp1 to regulate the FasL promoter. FasL has been implicated as a mediator of atherosclerotic plaque instability.80,81 Chronic vascular remodeling is sometimes associated with the formation of aneurysms. The therapeutic potential of inhibiting abdominal aortic aneurysm (AAA) formation in the rabbit was recently evaluated.82 Administration of decoy oligonucleotides encoding the binding sites for NF-κB and Ets-1 prevented the formation of AAA and is associated with a reduction in the expression of MCP-1, vascular cellular adhesion molecule-1, MMP-2, and MMP-9. A potential limitation of the decoy oligonucleotides is that the Ets-1 oligonucleotides may bind to other closely ETS factors such as Ets-2. The lack of specificity could, however, promote the therapeutic efficacy of this approach if closely related ETS factors exhibit similar functions in the same cell types. The use of RNA interference to block the expression of individual ETS factors could be used to define the role of closely related ETS family members in the development of AAA.

**Role of ETS Factors in Modulating Innate and Adaptive Immunity**

Vascular diseases are associated with both innate and immune responses. Selective members of the ETS family regulate genes involved in regulating genes involved in both innate and adaptive immunity (Figure 5). In response to endotoxin, the ETS factor ESE-1 is induced and regulates the expression of NOS2 and cyclooxygenase-2.59,60 Targeted disruption of the ETS factor PU.1 is associated with neutrophils that are defective in their ability to respond to chemokines, that fail to generate superoxide ions because of impaired expression of the NAD(P)H oxidase gp91phox (Nox2), and are ineffective at uptake and killing of bacteria.83 Targeted disruption of the ETS factor MEF leads to marked
reductions in the numbers and function of natural killer (NK) cells. Genes regulated by MEF include perforin, the chemokine IL-8, and interferon (IFN)-γ.84,85 MEF-deficient mice exhibit defects in both innate and adaptive immunity. Targeted disruption of Ets-1 is also associated with reduced numbers of natural killer (NK) T cells and diminished Th1-mediated T cell responses associated with profound decreases in the production of IL-2 and interferon-γ.86 Ets-1 functions as a cofactor, together with the T-box transcription factor T-bet, to regulate the expression of interferon-γ. Furthermore Th1 cells from Ets-1−/− deficient mice express the antiinflammatory cytokine IL-10 that is not normally expressed by these cells.87 Targeted disruption of the T-bet is similarly associated with changes in adaptive immunity that lead to marked reductions in the development of atherosclerosis when crossed with low-density lipoprotein receptor−deficient mice. Specific alterations in T-bet−/− deficient mice include a shift in T-helper cells toward a Th2 phenotype and a marked increase in the titer atheroprotective antibodies.88

Summary

In summary, the results of these studies suggest that several members of the ETS transcription factor family function as critical regulators of endothelial-specific gene expression during vascular development, and as regulators of angiogenesis associated with several diseases. ETS factors may function as critical effectors of several angiogenic growth factors. More recently, substantial evidence points toward an emerging role for their involvement as central regulators of vascular inflammation and immune function that are important in the pathogenesis of several human diseases associated with acute and chronic vascular inflammation. However, considerable gaps remain in our understanding of how the expression of selected ETS family members associated with inflammation are regulated, their exact involvement in regulating various stages of vascular inflammation and remodeling, and the specific target genes that are regulated by these factors. In addition, very little is known about posttranslational modifications of ETS factors, or additional proteins, including other transcription factors, that interact with ETS factors to modify their function in the setting of inflammation. Advances in proteomics and genomics will facilitate identification of the ETS-interacting proteins, posttranslational modifications, and additional downstream target genes. Several limitations exist among the techniques used in several of the published studies demonstrating specific roles for selected Ets family members in these processes. Targeted disruption of ETS family members may be associated with subtle developmental defects or alterations in immune function that could modulate inflammatory responses. Furthermore several different cell types may be affected. Future studies using conditional gain of function or loss of function in ECs or VSMCs may more clearly define the role of selected ETS family members that are independent of their broader role during cellular differentiation and development. The recent demonstration that tailored membrane permeable peptides can block the function of selected ETS transcription factors in vivo provides a novel method of testing the therapeutic potential in animal models of human disease associated with vascular inflammation, remodeling, or angiogenesis.44 Alternatively, the delivery of small interference RNA molecules, directed at inhibiting the expression of individual ETS family members, could be tested in vitro and in vivo. Finally, the recent advances in drug discovery and molecular modeling will enable the identified transcription factors to be considered as therapeutic drug targets through the identification of small molecules that can inhibit their function and be further developed and ultimately used one day to treat patients with diseases associated with vascular inflammation.

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References

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