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Regulation of Tissue Factor Gene Expression in Monocytes and Endothelial Cells: Thromboxane A_2 as a New Player

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Abstract

Tissue factor (TF) is the primary activator of the coagulation cascade. Under normal conditions, endothelial cells (ECs) and blood cells, such as monocytes, do not express TF. However, bacterial lipopolysaccharide (LPS) induces TF expression in monocytes and this leads to disseminated intravascular coagulation during endotoxemia and sepsis. A variety of stimuli induce TF expression in ECs in vitro, although it is unclear how much TF is expressed by the endothelium in vivo. LPS induction of TF gene expression in monocytic cells and ECs is mediated by various intracellular signaling pathways and the transcription factors NF-κB, AP-1 and Egr-1. In contrast, vascular endothelial cell growth factor (VEGF) induces TF gene expression in ECs via the transcription factors NFAT and Egr-1. Similarly, oxidized phospholipids (oxPAPC) induce TF expression in ECs and possibly monocytes via NFAT and Egr-1. Thromboxane (TX) A_2 can now be added to the list of stimuli that induce TF gene expression in both monocytes and ECs. Interestingly, inhibition of the TX-prostanoid (TP) receptor also reduces TF expression in ECs stimulated with tumor necrosis factor (TNF)-α and monocytes stimulated with LPS, which suggests that TP receptor antagonist may be useful in reducing pathologic TF expression in the vasculature.

Keywords

tissue factor; expression; thromboxane A2; endothelial cells; monocytes

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Introduction

TF is a transmembrane protein that functions as the primary initiator of the coagulation cascade[1]. Upon vascular damage, TF surrounding the vasculature comes into contact with blood. This leads to the formation of the TF:FVIIa complex that activates both FX and FIX, with subsequent thrombin generation, fibrin deposition and activation of platelets[1]. TF is constitutively expressed by cells within and surrounding the blood vessel wall, such as pericytes and adventitial fibroblasts[2,3]. It has been proposed that TF expressed by these cell types forms a hemostatic envelope that limits bleeding after vessel injury[2]. However, in pathologic conditions like sepsis, TF is also expressed by vascular cells, such as monocytes and ECs[4]. This expression can lead to disseminated intravascular coagulation (DIC) and thrombosis. TF expression by monocytes may be part of the innate immune response and is probably an attempt by the host to reduce the spread of pathogenic organisms. In atherosclerosis, TF is expressed by several cell types within atherosclerotic plaques, including macrophage-derived foam cells [5]. After plaque rupture, TF likely contributes to the formation of a thrombus.

TF expression in monocytes and ECs

Under normal conditions TF is not expressed by circulating blood cells[2]. However, one study found low levels of TF expression in a few CD14-positive monocytes[6]. Stimulation of monocytes and monocytic cells with LPS induces TF expression in vitro and in vivo[2,6–9]. Furthermore, we and others have shown that TF expression by hematopoietic cells contributes to the activation of coagulation in endotoxemic mice[10,11]. In vitro studies demonstrated that a variety of agonists, including LPS, IL-1β, TNF-α, thrombin and VEGF, induce TF expression on ECs[12–26]. In contrast, only a limited number of studies have reported TF expression by ECs in vivo. One study found co-localization of TF and the EC marker von Willebrand factor within the splenic microvasculature of septic baboons but not in ECs of pulmonary vessels[4]. Another study found TF protein on ECs in LPS treated mice and rabbits[27,28]. More recently, TF protein was observed on ECs at branch points of the aorta of septic baboons[29]. TF protein co-localized with fibrin deposition, suggesting that it was functional[29]. However, TF present on ECs was restricted to granular structures some of which were also positive for the leukocyte marker P-selectin glycoprotein ligand-1 (PSGL-1)[29]. This suggests that leukocyte-derived microparticles may deliver TF to activated ECs in vivo. In contrast to these studies, we and others did not detect TF expression by ECs in LPS treated mice, rats, and rabbits[30–33]. These different results may be caused by the relative sensitivity of the various techniques used to detect TF expression. Furthermore, it is possible that TF expression on ECs contributes to signaling rather than activation of coagulation. We analyzed the effect of EC-specific deletion of the TF gene on the activation of coagulation in mouse models of endotoxemia and sickle cell disease. We found that a deficiency of TF in ECs did not decrease the activation of coagulation in either model[34,35]. However, in the sickle cell disease model we found a reduction of IL-6 expression[35]. Similar results were observed with a FXa inhibitor or protease-activated receptor (PAR)-2 deficiency in non-hematopoietic cells suggesting that TF on ECs contributes to the induction of IL-6 expression via FXa activation of PAR-2.

Induction of TF gene expression in monocytes

i) LPS

The THP-1 cell line has been used as a model to study the regulation of TF gene expression in monocytes. These cells are derived from an acute human monocytic leukemia. LPS stimulation of THP-1 increases the rate of TF gene transcription, TF mRNA and TF protein. The human TF promoter contains a NF-κB site and two AP-1 sites in a distal region (Figure 1)[36]. In addition, the proximal region of the promoter contains two Sp1 sites (−172 to −112) and three overlapping Sp1/Egr-1 sites (−111 to +14) (Figure 1)[37]. The proximal region of the promoter (−170 to −59 bp) is required for basal expression[38].

An LPS response element (LRE) in the human TF promoter was identified by analyzing a series of plasmids containing different lengths of the promoter cloned upstream of the luciferase reporter gene. This element spans 56-bp (−227 to −172) and contains a NF-κB site and two AP-1 sites[36]. The NF-κB site is essential for full functionality of the LRE[36]. Interestingly, the NF-κB site does not match the κB consensus sequence due to a C instead of a G at position 1[39] and binds c-Rel-p65 heterodimers and not the prototypic p50-p65 heterodimers[40]. It was found that the transcriptional activation of the TF gene involves functional interactions between c-fos/c-jun and c-Rel-p65 heterodimers[14]. In addition, LPS induction of the TF gene was sensitive to nucleotide spacing between the proximal AP-1 and κB sites. Conservation of this 15-bp spacing in the human, murine, and porcine promoters may be required for physical association between c-fos/c-jun and c-Rel/p65 heterodimers[41]. Alternatively, the conserved spacing and defined DNA bending between the AP-1 and κB sites may be important for allowing the interaction of c-fos/c-jun and c-Rel/p65 with the TATA box binding protein and transcription factor IIB within the basal transcriptional machinery[41]. Additional studies showed that Egr-1 is required for maximal LPS induction of the TF promoter[42]. Mutation of the Egr-1 sites in the TF promoter or inhibition of the ERK 1/2 pathway, which induces Egr-1 gene expression, reduced the level of LPS induction of TF gene expression[42].

ii) Oxidized low-density lipoprotein (oxLDL)

We recently showed that oxLDL, but not LDL, increased TF expression in THP-1 and human peripheral blood mononuclear cells (PBMCs)[43]. Preincubation of the cells with a TLR-4 inhibitor (CLI-095) or simvastatin reduced the induction of TF expression[43]. We are currently analyzing the different signaling pathways and transcription factors that mediate oxLDL induction of TF expression.

Induction of TF gene expression in ECs

i) LPS, IL-1β and TNF-α

We found that LPS induction of TF gene expression in ECs was mediated by the LRE and Egr-1 sites (Figure 1), indicating that a common mechanism regulates TF gene expression in both human monocytes and ECs[14,42]. Furthermore, TNF-α and IL-1β also activated AP-1 and NF-κB in human umbilical vein ECs (HUVECs)[14]. A study that subjected human

pulmonary artery ECs to inhibitors of several intracellular signaling pathways demonstrated a critical role for protein kinase C (PKC) and for p38 in the induction of TF expression[44].

ii) CD40L

CD40L induces TF expression through a variety of pathways in ECs, ultimately involving the transcription factors AP-1, NF-κB and Egr-1 that all appear to be necessary in order to achieve a maximal response (Figure 1)[19–22].

iii) Antiphospholipid antibodies

Antiphospholipid syndrome is an autoimmune disease caused by antiphospholipid antibodies. Patients are hypercoagulable particularly during pregnancy[45,46]. Anti-phospholipid antibodies have been shown to induce TF expression on HUVECs through an unknown receptor but involving the NF-κB and p38 intracellular pathways (Figure 1)[24]. Similar results were observed using PBMCs[47]. Induction of TF expression in monocytes and ECs may explain the prothrombotic state caused by these antibodies and it may lead to the development of more directed antithrombotic/anti-inflammatory therapy in these patients, for example by inhibition of p38 (Figure 1).

iv) VEGF

VEGF has been shown to induce TF gene expression in HUVECs via two distinct pathways. First, it triggers NFAT dephosphorylation by calcineurin, which allows nuclear translocation of NFAT, binding to a site in the TF promoter (−197 to −183) and induction of TF gene expression[16]. There is some evidence that it also increases the transcriptional activity of AP-1[16,48]. Secondly, VEGF induces TF gene expression via a PKC-dependent pathway that leads to activation of ERK 1/2 and Egr-1 gene expression[17]. Importantly, NFAT and Egr-1 synergistically cooperate in VEGF induction of the TF promoter (Figure 1)[49].

v) oxPAPC

OxLDL and oxPAPC induce TF expression in HUVECs[50]. Interestingly, oxPAPC induction of TF gene expression involved both NFAT and Egr-1 in a similar manner to VEGF (Figure 1)[18].

vi) Shear stress

Two studies have reported that induction of the TF gene in ECs by laminar shear stress was mediated by a GC-rich region (−111 to +14) containing three copies each of the Egr-1 and Sp1 sites[15,51]. These Egr-1 and Sp1 binding sites are overlapping which precludes binding of both transcription factors at the same time[15]. One study concluded that the induction was mediated by modifying Sp1 bound to the promoter[51]. However, a second study concluded that shear stress induced the expression of Egr-1 and that this leads to increased TF gene expression (Figure 1)[15], which is a more plausible mechanism.

vii) Indolic uremic solutes

Uremic solutes are increased in patients with chronic kidney disease and could contribute to their prothrombotic phenotype and high cardiovascular mortality. Recently, a study reported

that the indolic uremic solutes indoxyl sulfate and indole-3-acetic acid induce TF expression in HUVECs[23]. Interestingly, this induction was mediated by the aryl hydrocarbon receptor (AHR) (Figure 1)[23]. After activation, AHR translocates to the nucleus and acts as a transcription factor. However, there is no consensus sequence for AHR binding in the TF promoter, although it may bind to a non-consensus sequence. Alternatively, AHR may enhance signaling pathways or interact with transcription factors that regulate TF gene expression[52–54].

A summary of the different intracellular signaling pathways and transcription factors involved in the induction of TF gene expression in monocytes and ECs is shown in Table 1.

TXA_2 and TF expression in monocytes and ECs

The eicosanoid TXA_2 is a proinflammatory mediator. It activates a variety of cell types, including monocytes and ECs, by binding to the TP receptor[55]. A paper in this issue of Vascular Pharmacology found that a TP receptor agonist (U46619) induced TF expression in ECs[25]. A previous study showed that U46619 induces MCP-1 expression in ECs[56]. The TP receptor activates a PKC dependent pathway that leads to the activation of AP-1 and NF-κB[56]. In a mouse model of microcirculatory dysfunction in the liver, TNF-α induced leukocyte adhesion was significantly reduced by administration of a TXA_2 synthase inhibitor (OKY-046) and in TP receptor knockout mice, suggesting TP receptor signaling may promote hepatic dysfunction elicited by TNF-α[57]. The phenotype of TP deficient mice was more pronounced than that of TX synthase deficient mice suggesting that ligands other than TXA_2 may activate the TP receptor[55]. This study indicated that TXA_2 stimulation of the TP receptor contributes to the effects of TNF-α in vivo. Interestingly, Del Turco and colleagues found that inhibition of the TP receptor reduced TNF-α induction of TF expression in ECs[25]. Importantly, TXA_2 production is enhanced in HUVECs by TNF-α or platelet-activating factor (PAF) stimulation[58–60]. However, Del Turco and colleagues concluded that the reduction of TNF-α induction of TF expression by blocking the TP receptor was not due to the production of TXA_2 or prostanoids by the ECs since they did not observe any effect after treating the cells with acetylsalicylic acid (ASA) or indomethacin[25]. One concern is that levels of the TXA_2 metabolic product TXB_2 were only measured at 24 hours. Moreover, the cells may express other ligands that activate the TP receptor[55].

An alternative explanation for the effect of the TP antagonist on TNF-α is that the activated cells express TXA_2 and this activates the TP receptor and enhances the induction of TF expression (Figure 2). If this notion is correct one would predict that the effect of the TP antagonist would be more pronounced at later times. Unfortunately, Del Turco and colleague only analyzed TF expression at 6 hours[25]. Another study found that TNF-α or PAF induction of ICAM-1 expression in ECs was decreased with a TXA_2 synthesis inhibitor (DP-1904)[58]. Similarly, treatment of ECs with a TP receptor antagonist (SQ29 548 or BAYu3405) reduced TNF-α or PAF induction of ICAM-1 and MCP-1 expression[56,59]. Taken together, these results suggest that TNF-α and PAF stimulation of ECs leads to production of TXA_2 that is secreted and then activates intracellular pathways through the TP receptor (Figure 2).

Consistent with the above results in ECs, TF expression is reduced in LPS stimulated human monocytes by a TP receptor antagonist (SQ29 548) and by indobufen, a cyclooxygenase (COX)-1/2 inhibitor, which decreases TXA_2 production[61,62]. Treatment with ASA, a COX-1 inhibitor, does not reduce TF expression, suggesting that COX-2 metabolites, such as TXA_2, are regulators of TF expression[61]. Indobufen also led to reduced ERK 1/2 phosphorylation, suggesting an involvement of this pathway in induction of TF expression[61]. In another study examining the effect of a variety of inhibitors on the LPS induced monocyte TF expression in human whole blood, the TP receptor and PAF receptor were shown to be necessary for full induction of TF activity[63].

Conclusions

TF is a cellular receptor that initiates blood coagulation. It is constitutively expressed in some extravascular cell types and its expression is inducible in several vascular cell types, including monocytes and ECs. Further studies are needed to clarify the exact mechanism of TNF-α induced TP receptor activation and to assess the effects of this activation in different cell types and in vivo in different pathologic settings. The observation that the TP receptor is an important inducer of TF expression in ECs is intriguing because antagonization of the TP receptor may represent a new treatment of acute and chronic inflammatory conditions that involve TF expression, such as sepsis and atherosclerosis. Terutroban, the TP receptor antagonist used by Del Turco and colleagues has already been compared to ASA in a randomized controlled trial (PERFORM)[64] on patients with recent ischemic stroke or transient ischemic attacks. No significant difference was found for the primary endpoint which was a composite of fatal or non-fatal ischemic stroke, fatal or non-fatal myocardial infarction, or other vascular death. One possible explanation for the negative result, with the notion that a major effect of the drug is the inhibition of TF expression, is that there is little benefit to be gained after the ischemic event. It would be interesting, however, to see TP receptor antagonists evaluated in the primary prevention of stroke or coronary artery disease and in the treatment of DIC or other thrombotic conditions associated with monocyte TF expression.

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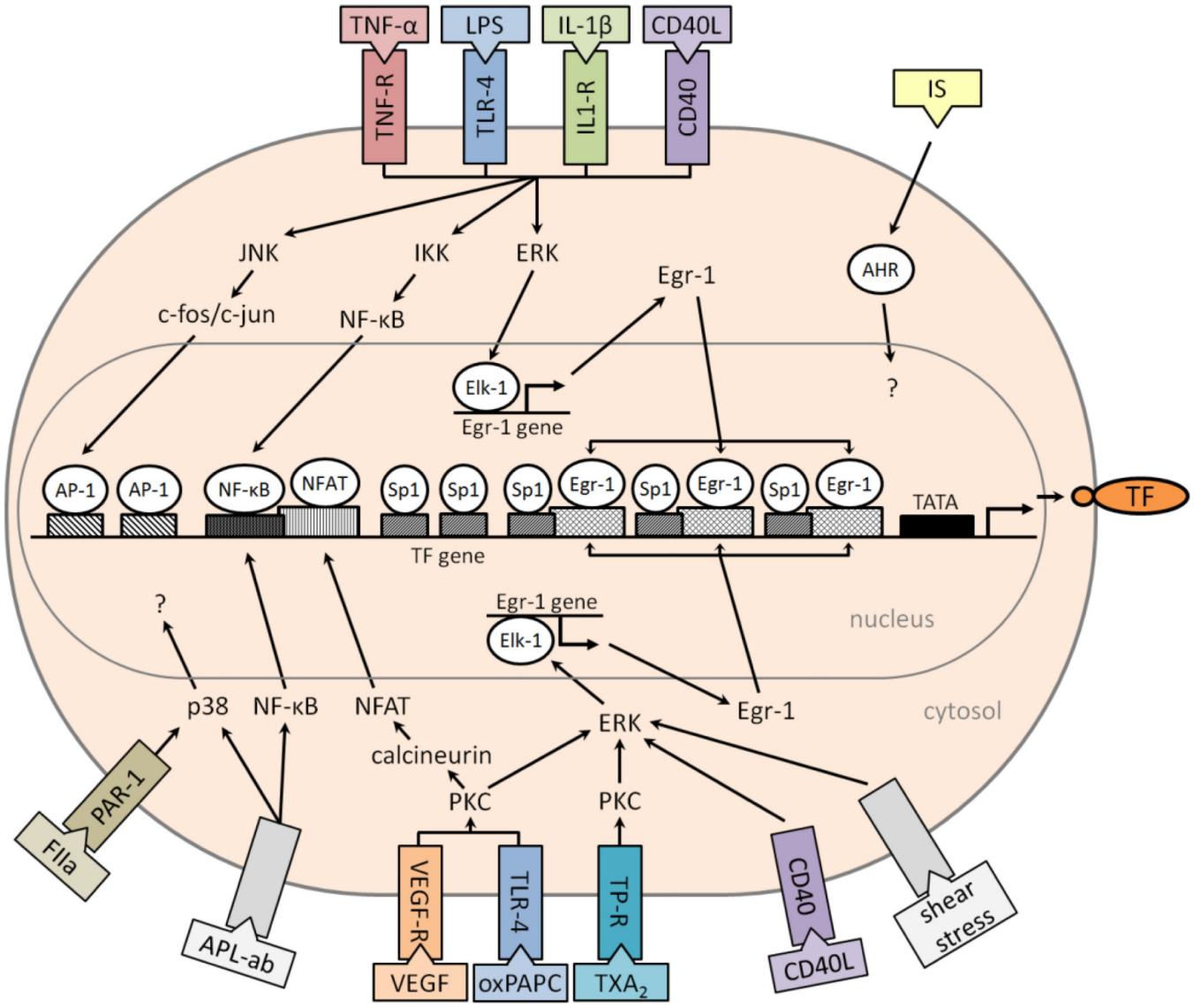

Figure 1.

Induction of the human tissue factor (TF) promoter in endothelial cells. Shown are intracellular signaling pathways, transcription factors and DNA binding sites that regulate TF gene expression in response to different agonists. Receptor (R), oxidized phospholipids (oxPAPC), antiphospholipid antibody (APL-ab), aryl hydrocarbon receptor (AHR), thromboxane A_2 (TXA_2), thrombin (FIIa), indoxylsulfate (IS), vascular endothelial growth factor (VEGF), protease-activated receptor 1 (PAR-1).

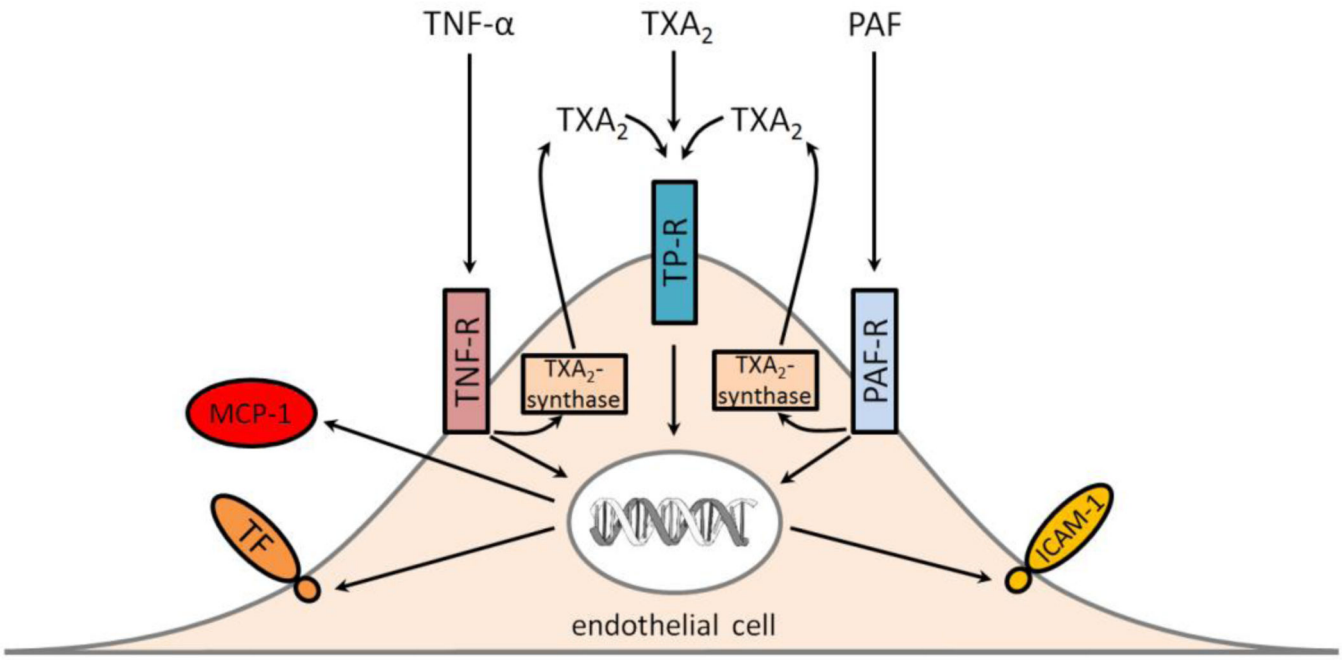

Figure 2.

Proposed mechanism by which the TP receptor (TP-R) contributes to gene expression in endothelial cells. TP-R can directly be activated by TXA_2 or receptor agonists and induce the expression of tissue factor (TF), ICAM-1 and MCP-1 In addition, the presence of the TP-R enhances TNF-α and PAF induction of gene expression by increasing TXA_2 expression by TXA_2-synthase. TNF-α receptor (TNF-R), PAF receptor (PAF-R).

Table 1

TF gene expression in monocytes and endothelial cells Monocytes

Monocytes					
Agonist	**Signaling pathways**	**Transcription factor**	**Promoter region**	**Cell type**	**References**
LPS		AP-1, NF-κB	−227 to −172	THP-1	36,40
LPS	MEK 1/2, ERK 1/2, Elk-1	Egr-1	−111 to +14	THP-1	42
LPS		AP1, NF-κB, Egr-1	−227 to −172, −111 to +14	THP-1	38
LPS	p38; ERK 1/2			THP-1	65
oxLDL				THP-1, PBMC	43
IS, IAA		AHR		PBMC	23
APL Ab	p38	NF-κB		THP-1	47
TXA2	ERK 1/2			PBMC	61,63

Endothelial cells					
Agonist	**Signaling pathways**	**Transcription factor**	**Promoter region**	**Cell type**	**References**
LPS, TNF-α, IL-1β		AP-1, NF-κB	−227 to −172	HUVEC	14
Thrombin	PKC, p38			HPAEC	44
Shear stress		Egr-1	−111 to +14	HUVEC	15
VEGF	calcineurin	NFAT	−197 to −183	HUVEC	16
VEGF	PKC, ERK 1/2	Egr-1		HUVEC	17
oxPAPC	PKC, ERK 1/2; calcineurin	Egr-1; NFAT		HUVEC	18
CD40L		AP1, NF-κB, Egr-1	−278 to +121	HSVEC, HUVEC	22
IS, IAA		AHR		HUVEC	23
APL Ab	p38	NF-κB		HUVEC	24
PAF				HUVEC	66
TXA2	PKC, ERK 1/2, JNK			HUVEC	25

Bacterial lipopolysaccharide (LPS), oxidized low-density lipoprotein (oxLDL), peripheral blood mononuclear cell (PBMC), indoxyl sulfate (IS), indole-3-acetic acid (IAA), aryl hydrocarbon receptor (AHR), antiphospholipid antibody (ABL Ab), thromboxane A2 (TXA2), human umbilical vein endothelial cell (HUVEC), human pulmonary artery endothelial cell (HPAEC), vascular endothelial growth factor (VEGF), oxidized phospholipids (oxPAPC), human saphenous vein endothelial cell (HSVEC), platelet activating factor (PAF)