Endothelial Cell Gene Regulation

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Endothelial cells (ECs) display phenotypic heterogeneity. Endothelial cell heterogeneity is mediated, at least in part, by site-specific and time-dependent differences in gene transcription. The goal of this review is to provide a conceptual framework for approaching EC gene regulation in the adult vasculature. We summarize data from cell culture studies that provide insight into the transcription factors involved in mediating gene expression in ECs. Next, we review the results of in vivo studies relating to gene regulation in the intact endothelium. Finally, we draw on both the in vitro and in vivo results to propose a model of gene regulation that emphasizes the importance of the extracellular environment in controlling site- and time-specific vascular gene expression. (Trends Cardiovasc Med 2005;15:174.e1–174.e24) © *2005, Elsevier Inc.*

The endothelium lines the vasculature and is involved in multiple homeostatic functions including the control of vasomotor tone, the trafficking of cells and nutrients, the maintenance of blood fluidity, and the growth of new blood vessels (Cines et al. 1998). Importantly, these properties vary both in space and time, giving rise to the phenomenon termed endothelial cell (EC) heterogeneity (reviewed in Aird 2003a).

There are several reasons for studying transcriptional regulation in the endothelium. First, the phenotype of the endothelium is dictated in large part by space and time-specific differences in gene expression. Therefore, the elucidation of mechanisms of transcriptional control should provide important insights into the molecular basis of vascular diversity. Second, transcriptional networks act as signal transducers in the endothelium, coupling input to output. Thus, transcription factors hold a key to understanding the link between microenvironment and cellular phenotype. Third, each transcription factor is coupled to multiple upstream signals and downstream target genes. Therefore, the characterization of these so-called hubs in the context of network topology will provide not only important mechanistic information, but also a powerful foundation for targeted therapy (Aird 2003b).

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• Defining Endothelial Cells

An interesting question is how one defines an EC. From an anatomic standpoint, ECs are readily described by their location on the luminal side of the blood vessel wall. A developmental biologist might argue that ECs are defined by one or a small number of lineage switches that occur during embryogenesis. Ultrastructural properties that are classically associated with the endothelium, such as Weibel–Palade bodies and vesicular–vacuolar organelles, are not expressed uniformly throughout the vascular tree. Other properties such as caveolae are not unique to the endothelial lineage. From the standpoint of molecular markers, few, if any, EC-specific transcripts or proteins are expressed in all ECs. Functionally, the endothelium displays remarkable division of labor. For example, most leukocyte trafficking takes place in post-capillary venules; endothelial-mediated vasorelaxation is a property of arterioles; barrier properties normally vary throughout the vasculature; and hemostatic balance is mediated by vascular bed-specific patterns of natural anticoagulants and procoagulants. Finally, in transgenic mouse assays, most EC-specific promoters contain information for expression in discrete subpopulations of ECs. To summarize, each definition—with the exception of anatomic location—falls short of fully capturing the endothelium.

• Phenotypic Heterogeneity of the Endothelium

When considering the remarkable degree of heterogeneity of the endothelium, two questions arise: (a) why are there so many differences between endothelial phenotypes, and (b) how are these differences generated and maintained? The first question requires an evolutionary explanation; the second calls for a proximate mechanism.

Several selective forces may explain the existence of endothelial heterogeneity. First, the endothelium must meet the diverse needs of the underlying tissue environment. For example, fenestrations within glomerular endothelium facilitate filtration function of the kidney; the tight junctions and enzymatic machinery of the blood–brain barrier protect this organ from fluctuations in blood composition; and the fenestrae and lack of organized basement membrane in hepatic sinusoids enable ready passage of substrates from nutrient-rich portal venous blood to underlying hepatocytes. Second, the endothelium must cope with different environments. For example, the endothelium of lung capillaries is exposed to high levels of oxygen, whereas the endothelium of the vasa recta in the inner medulla of the kidney is exposed to a profoundly hypoxic, hyperosmolar, and hyperkalemic tissue

microenvironment. Although little is known about the latter blood vessels, their endothelium must be uniquely adapted to withstand such extreme conditions. Finally, when outer epithelial layers are breached by pathogens, the endothelium plays a key role in orchestrating the host innate immune response. The greater the heterogeneity and adaptability the more poised the endothelium is to deal with the unpredictable properties of the outside world, particularly those arising from the host–pathogen arms race.

From a mechanistic standpoint, endothelial heterogeneity arises from cues within the extracellular environment. Each EC in the human body is analogous to a miniature adaptive input–output device (Figure 1). Extracellular input is coupled to output by a highly intricate array of nonlinear signaling pathways, which often begin at the cell surface and converge at the level of gene transcription.

Signal input varies from one site of the vascular tree to another and from one moment to the next. For example, capillary ECs in the heart are exposed to unique regional biomechanical forces as well as cardiomyocyte-derived paracrine mediators, whereas the endothelium of the blood–brain barrier receives input from surrounding astroglial cells. As input varies in space and time, so does endothelial output or phenotype.

In addition to these reversible effects of the microenvironment, extracellular signals may also induce epigenetic modification of ECs. As a result, certain site-specific properties become irreversibly "locked in" and mitotically stable. These events are particularly important during embryogenesis, where they contribute to such fundamental processes as arterial–venous identity.

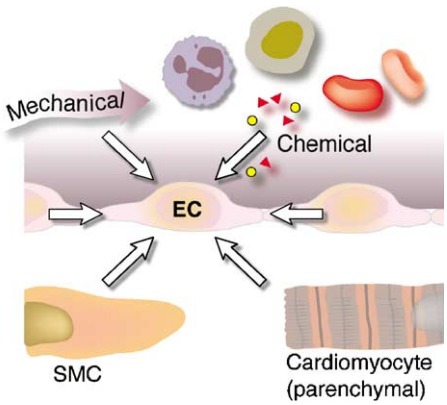

Figure 1. *Endothelial cell as an input–output device*. Each EC senses biochemical and biomechanical signals from the surrounding microenvironment. Signals may originate from blood vessel lumen, neighboring ECs, and/or abluminal surface. Shown (arrows) are (bio)mechanical input and (bio)chemical input. Chemical input may consist of paracrine signals derived from circulating blood cells, neighboring ECs, vascular smooth muscle cells (SMCs), and parenchymal cells (e.g., cardiomyocyte); or soluble signals that function over a distance (e.g., hormones, lipoproteins, pH, oxygen). For purposes of illustration, the arrows all point in one direction toward the EC. However, the dialogue between the EC and its environment is bidirectional. Not shown are the intracellular signal transduction networks or the cellular output/phenotype.

• Classification of Endothelial Cell Genes

Endothelial cell genes may be classified according to whether they are constitutively or inducibly expressed, whether they are specific or not to ECs, and whether they are expressed throughout the endothelium or in subsets of ECs (Figure 2).

A distinct advantage of studying genes which are specifically and constitutively expressed in ECs is that an understanding of their transcriptional control may provide important insights into the molecular basis of lineage determination. Moreover, the information derived from these studies may be useful for designing cell-type-specific targeting strategies for treatment of vascular diseases.

However, the intrinsic value of an endothelial gene, either as a functional entity or as a target of investigation, does not necessarily correlate with its degree of cell-type specificity. For example, E-selectin is expressed exclusively in ECs, whereas intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 are also expressed in other cell types. There is no evidence that E-selectin is more important in mediating cell adhesion than its non-cell-type-specific counterparts. As another example, tissue factor is not normally expressed in the intact endothelium, but is expressed by monocytes, fibroblast, and many parenchymal cells.

	SPECIFICITY	INDUCIBILITY	LOCALITY
Gene A	EC	Inducible	Vasc. bed
Gene B	EC	Inducible	All-endo
Gene C	EC	Constitutive	Vasc. bed
Gene D	EC	Constitutive	All-endo
Gene E	Non-sp.	Inducible	Vasc. bed
Gene F	Non-sp.	Inducible	All-endo
Gene G	Non-sp.	Constitutive	Vasc. bed
Gene H	Non-sp.	Constitutive	All-endo

Figure 2. *Schematic of classes of EC genes*. Endothelial cell genes may be classified according to specificity, inducibility, and locality. Under the heading "specificity," genes are either specific to ECs (EC), or expressed in other cell types as well (Non-sp., nonspecific). Under the heading "inducibility," genes are either inducible (or repressible) by signals within the extracellular microenvironment, or they are constitutively expressed (constitutive). Locality refers to whether the gene is expressed in distinct subsets of ECs/vascular beds (Vasc. bed), or is pan-endothelial in its distribution (All-endo). Examples of class A genes include E-selectin, thrombomodulin, endothelial protein C receptor, eNOS, endocan, and vWF; class B genes unknown; class C genes include tissue factor pathway inhibitor, receptor tyrosine phosphatase μ, Ephrin B2, Ephrin B4; class D genes include PECAM-1 (CD31) and VE-cadherin; class E genes include VCAM-1, P-selectin, tissue factor, Egr-1; class F genes unknown; class G genes include P-glycoprotein, GLUT4; class H genes include β-actin and other "housekeeping" genes. The divisions between classes are not absolute—for example, there is increasing evidence that many of the constitutive genes are in fact modulated by the microenvironment. Moreover, there is a question as to whether there is any EC-specific gene that is expressed in *every* EC of the body. Finally, many genes in class A are expressed in a limited number of other cell types.

Endothelial expression of tissue factor has been reported in certain pathologic conditions, including tumors (Contrino et al. 1996), atherosclerosis (Crawley et al. 2000), sickle cell anemia (Solovey et al. 2004), sepsis (Drake et al. 1993), and cardiac allograft rejection (Nagayasu et al. 2000). It is possible that under these conditions endothelial tissue factor plays an important pathogenic role and that an understanding of its transcriptional regulation would provide new insights into mechanisms of disease.

In summary, the site- and time-specific phenotype of an EC is dictated by its full complement of transcripts and proteins, and not simply by its

Table 1. Examples of agonist-mediated induction/repression of transcription factors and downstream target genes in EC[a]

Agonist	Transcription factor	Target genes/cell function [b]
Oxygenation		
Hypoxia	AP-1	HO-1
		eNOS
	Egr-1	–
	Elk-1	–
	HIF	CRLR
		Endoglin
		MDR-1
		SDF-1
	HIF+GATA-2+AP-1	ET-1
	NERF-2	TIE-2
	Smad2/3	TGF-β
	Sp1	MDR-1
Hemodynamic forces		
Laminar shear stress	GATA-6	Inhibits u-PA
	KLF2	–
	Negative SSRE binding protein	Inhibits Tie-1
	c-Myc	MMP-9
	NF-κB	ICAM-1
		eNOS
	Inhibits NF-κB	Inhibits PDGF-B
	GR	–
	Sp1	Flk-1
	Inhibits Sp1	Inhibits P2X4
	SREBP	–
	AP-1	eNOS
	Nrf2	NQO1, HO-1
	CRE binding protein	COX-2
Cyclical strain	AP-1	ET-1
Stretch	NF-κB	IL-6
Chemokines and cytokines		
IFN-α	STAT-4	MCP-1, SOCS3
IFN-γ	STAT-1	CD40
		TAP-1, IRF1, Class 1 MHC
IL-1	AP-1, Sp1	ICAM-1
	NF-κB, C/EBP	iNOS
IL-4	STAT-1	MCP-1
	AP-2, NF-1, Sp1, STAT-6, but not NF-κB or AP-1	–
IL-6	STAT-3	–
IL-10	STAT-3	eNOS
IL-18	NF-κB	IL-1, TNF-α
LIX	NF-κB	IL-1, TNF-α
PAF	STAT-3	–
TNF-α	ATF3	Cell death
	CREB	–
	E2F1 inhibition	Inhibits E2F1
	Id1, Id3	E-selectin, not ICAM-1
	IRF-1	VCAM-1
	NF-κB	E-selectin, TF
		Fractalkine
		IL-8
		MadCAM
		MCP-1, IL-8, ICAM-1, VCAM-1, E-selectin
		TF
	NUR77	PAI-1
	Sp1	Fractalkine
	Sp1, Sp3 inhibition	Inhibits eNOS
TNF-α+IFN-γ	STAT-1, IRF	CD40
HMGB1	NF-κB, Sp1	–
Growth factors, hormones, glucose		
AGE	NF-κB, AP-1	VEGF
Ang-1	Forkhead	–
Dihydrotes tosterone	Androgen receptor/ NF-kB	VCAM-1
ET-1	Ets-1	–
FGF-2	Tcf-4	Cyclin D1
	STAT-3	–
	Egr-1	
Glucose	AP-1	Fibronectin
		IL-8
	Egr-1	Flt-1, PAI-1
	NF-κB	VCAM-1
		eNOS inhibits cell migration
	Fibronectin	
GM-CSF	NF-κB	–
HGF	Forkhead	–
	Ets-1	MMP-1, HGF, c-met
	Egr-1	ACE
IGF-1	RUNX2	Tube formation
Insulin	AP-1	eNOS
	Egr-1	Flt-1, PAI-1
Leptin	AP-1	ET-1
	STAT-3	VEGF
Placental growth factor	AP-1	–
Prolactin (16-kD N′ terminal fragment)	NF-κB	Caspases 3, 8, 9; E-selectin
Relaxin	NF-κB	ET_B
VEGF	Ets-1	Neuropilin, angiopoietin-2, cell proliferation
		Angiopoietin-2
	NF-AT	DSCR-1
		Proliferation
		DAF
	GATA	DSCR-1
		COX-2
	Forkhead	$p27^{kip1}$
		MnSOD
	NF-κB	MnSOD
		TF
	STAT-3	VEGF
	Egr-1	TF, Flt-1, u-PA
	Id1, Id3	ICAM-1, but not E-selectin; EC proliferation
	SREBP1/2	–
	Sp1	KDR
	Vezf1	VEGF-mediated proliferation, migration, and tube formation
Thymosin β4	AP-1	PAI-1
Lipoproteins and related mediators		
Cholesterol	AP-1	ICAM-1
Diabetic LDL	STAT-5B	p21 and G1 fraction
Native LDL	LXR	ABCA1
oxHDL	NF-κB	–
oxLDL	ATF3	Cell death

Table 1. continued

Agonist	Transcription factor	Target genes/cell function [b]
Lipoproteins and related mediators		
VLDL, oxVLDL	CREB, NF-κB	–
LPL+VLDL	PPARα	Ac-CoA-oxidase
LPA, S-1-P	NF-κB	ICAM-1
Fatty acids	Sp1	Heparanase
Oleic acid	NF-κB	ET-1
5(S)-HETE	STAT-3	–
Oxidized phospholipids (OxPAPC)	STAT-3	IL-8
		TF
	NFAT, Egr-1	
	CREB	HO-1
	Egr-1	–
Epoxyeicosatrienoic acids (EET)	CRE	t-PA
Linoleic acid	NF-κB, AP-1	–
ω-3 fatty acids	PPAR-α	–
Matrix and related factors		
Fibronectin	NF-κB	E-selectin, VCAM-1, ET-1, CYR-61
Three-dimensional collagen type-1	GATA-2	MMP-2
Type 1 collagen	Ets-1	MMP-1
TGF-β	Smad2	eNOS
	Smad1/2/5, Id	Cell migration
	CREB	RANKL
	Smad3/4, AP-1	ET-1
	Smad6	Inhibits TM
Serine proteases		
Thrombin	AP-1	Prepro-ET
	dbpB	PDGF-B
	Egr-1	–
	NF-κB	ICAM-1
	NF-κB, GATA-2	VCAM-1
	NF-AT	DSCR-1
	Sp1	KDR
	SRF	Egr-1
Cell–cell interactions		
Platelet-EC	NF-κB	MCP-1, VCAM-1
Neutrophil-EC	Ets-1	Tube formation
Neutrophil-derived proteases	NF-κB (cleaves and inhibits)	
Vasomotor tone		
NO	Inhibits NF-κB	Inhibits ET-1
	Nrf2	HO-1
Ang II	AP-1	ET-1
	NF-κB	VCAM-1, ICAM-1
ANP	AP-1	HO-1
Pathogens		
Orienta tsutsugamushi	AP-1, NF-κB	MCP-1
Chlamydia pneumoniae PMP	NF-κB	IL-6, MCP-1
Chlamydia pneumoniae infection	NF-κB	ICAM-1
Shiga toxin	NF-κB	MCP-1, IL-8
African swine fever virus (infection)	Decreases p65 NF-κB	Inhibits IL-8, E-selectin, but increases TF
Bartonella henselae (infection)	NF-κB	ICAM-1, E-selectin
Bartonella bacilliformis infection	AP-1	–
EBV infection or LMP-1 overexpression	NF-κB	–
HIV protein Tat	NF-κB	E-selectin
HIV pcTat	NF-κB	Monocyte adhesion
HIV Tat1-72	NF-κB, AP-1	–
HHV-8 (retroviral infection of ORF74)	NF-κB	–
Drugs/toxins		
Arsenite	NF-κB, AP-1	–
Bleomycin	Egr-1	ACE
	NF-κB	E-selectin
	NF-κB, Nrf-1/2	γ-Glutamyl-cysteine synthetase
Cocaine	NF-κB, AP-1	–
Doxorubicin	p53, not NF-κB	CD95
	NF-κB	Cell apoptosis
HMG-Co reductase inhibitors	Inhibits NF-κB, AP-1, HIF-1α	–
Isoproterenol	NF-κB	IL-18
Drugs/toxins		
Methamphetamine	NF-κB, AP-1	TNF-α
Disease-related mediators		
Human lymphoma-derived soluble factors	NF-κB	Leukocyte adhesion
Amyloid-β peptide	AP-1	Bim
Anti-phospholipid antibodies	NF-κB	–
Homocysteine	NF-κB	Fas
MetHb	NF-κB	E-selectin, IL-6, IL-8

See online supplement for references, endothelial cell types used, and full list of abbreviations.

[a] Data are not all-inclusive, but rather are representative of published studies over the past 4 years. Studies of lymphatic EC are not included. The results (as shown in the table and, in most cases, in the original papers) do not discriminate between primary and secondary effects of transcription factors. For example, many of the transcription factors listed under hypoxia my lie downstream of HIF-1α. The reader should refer to the original paper for passage number, agonist dose(s), treatment time(s), and the rigor of assays establishing connection between transcription factor and target gene (e.g., promoter analysis, chemical inhibitor, antisense oligonucleotides, siRNA).

[b] Induction of gene expression unless otherwise stated.

repertoire of lineage-specific genes. Indeed, a full understanding of the mechanisms by which differential gene expression determines phenotypic heterogeneity requires a consideration of both cell-type-specific and non-specific gene classes.

- **Endothelial Cell Gene Regulation In Vitro**

To date, studies of constitutively expressed, cell-type-specific genes (Figure 2,

classes C and D) have provided relatively few insights into the biology of adult endothelium. As a general rule, lineage-specific gene expression in adult ECs is mediated by combinatorial interactions between non-cell-type-specific transcription factors, including Sp1, GATA proteins (GATA-2, GATA-3, and GATA-6), and the Ets family of transcription factors (Ets-1, Ets-2, ELF-1, NERF2, and Fli-1). A possible exception is the transcription factor, Vezf1, which is restricted in its expression to ECs (Xiong et al. 1999), and has been shown to mediate expression of endothelin-1 (Aitsebaomo et al. 2001). In contrast to other lineages, such as erythroid cells, hepatocytes, and skeletal myocytes, the endothelium does not seem to possess a cell-type-specific master switch that mediates differentiation and/or expression of multiple differentiation markers. However, it is formally possible that such factor(s) exists but awaits discovery. Perhaps lineage-specific transcription factors operate transiently during endothelial differentiation and/or angiogenesis, but are no longer necessary for regulating target gene expression in mature endothelium of preexisting blood vessels. Alternatively, ECs possess yet-to-be-identified cell-type-specific co-activators that account for lineage-restricted gene expression.

Compared with the paucity of data relating to cell-type-specific gene regulation, there is a wealth of knowledge regarding modulatable gene expression (Figure 2, classes A, B, E, and F). Many extracellular signals have been shown to alter gene transcription in cultured ECs (Table 1). Unfortunately, differences in study design (e.g., cell type, passage number, and assay) hinder comparisons of the published data (Figure 3). These differences notwithstanding, several themes emerge from the in vitro studies. First, the various agonists activate overlapping yet distinct patterns of transcription factors and downstream target genes. In other words, EC activation is not an all-or-none phenomenon. Second, input is typically transduced by non-cell-type-specific transcription factors. Finally, the collective data from cell culture studies point to the truly remarkable capacity of ECs to recognize and respond to extracellular signals.

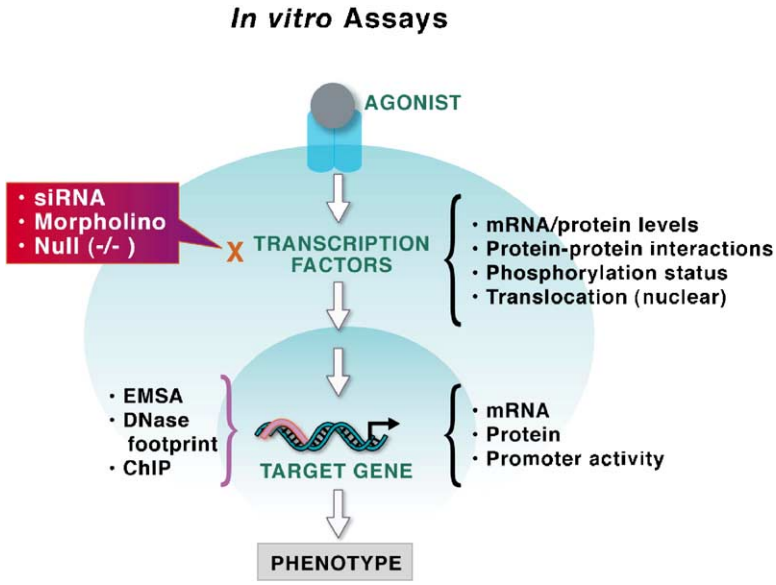

Figure 3. *In vitro assays for studying EC gene regulation*. Shown is an agonist binding to receptor in the surface of EC. Activation of receptor leads to changes in transcription factor(s) and downstream target genes. Assays at the level of transcription factor include mRNA or protein levels, interactions with other transcription factors and/or co-activators, phosphorylation or dephosphorylation, and/or nuclear exclusion/translocation. Binding of transcription factor to DNA may be assayed by electrophoretic mobility shift assay (EMSA), DNase footprint, or chromatin immunoprecipitation (ChIP). Target gene readouts include mRNA and protein levels as well as promoter–reporter gene activity, and cellular function. The above assays may be carried out in wild-type ECs, in cells null for one or another transcription factors, or in cells that have been transfected with siRNA or morpholino antisense oligonucleotides.

• Endothelial Cell Regulation In Vivo

Well before the routine isolation of ECs, electron microscopic studies demonstrated clear differences in phenotype between vascular beds, perhaps the most striking example being the tight barrier properties of the blood–brain barrier (Reese and Karnovsky 1967). If one believes that such phenotypes are controlled at the level of gene transcription, then these differences in ultrastructure provided an early hint for the existence of differential gene expression. These observations were supported by immunohistochemical and in situ hybridization studies in the 1980s and 1990s, demonstrating regional variation in gene and/or protein expression within different vascular beds (Kumar et al. 1987, Page et al. 1992, Turner et al. 1987). More recently, sophisticated proteomic approaches have revealed extensive heterogeneity of the endothelium in vivo (Arap et al. 2002, Oh et al. 2004).

The availability of cell culture techniques in the mid-1970s marked the beginning of a new era in endothelial biology, one that saw a striking increase in the number of publications in the field (Nachman and Jaffe 2004). Endothelial cell cultures offer a powerful tool for dissecting molecular mechanisms of transcriptional regulation. However, because ECs undergo phenotypic drift when removed from their native environment, these assays do not lend themselves to a molecular dissection of site-specific regulatory mechanisms.

Perhaps the first evidence for regional differences in mechanisms of transcriptional regulation was provided by early standard transgenic approaches in the late 1980s and 1990s. The first reports demonstrated that a small fragment of the von Willebrand factor (vWF) promoter, or the *Tie-2* gene, directed expression of a reporter gene to limited subpopulations of ECs in the intact animal (Aird et al. 1995, Schlaeger et al. 1995). In each case, the addition of further 5′ and/or 3′ DNA sequences resulted in more widespread expression in the endothelium (Aird et al. 1997, Schlaeger et al. 1997). Many

Table 2. Promoter analysis in transgenic mice

Gene	*Assay*	*Promoter (species)*	*Reporter gene*	*Pattern of expression*	*Non-EC*
Activin receptor-like kinase 1	Stable transgenics	Various promoter fragments (mouse)	*LacZ*	9.2-kb promoter directs arterial specific expression in adults	
CD34	Stable transgenics	160-kb (human)	Human CD34	ECs in many vascular beds in adult	Bone marrow
c-mpl	Stable transgenics	2-kb (mouse)	Human placental alkaline phosphatase	Some ECs in blood vessels of embryo (and yolk sac); none in adult	Megakaryocytes
eNOS	Stable transgenics	1.6-kb (human)	*LacZ*	Expression in ECs of brain, heart, skeletal muscle, and aorta. β-Galactosidase activity was consistently absent in vascular beds of the liver, kidney, and spleen	
	Stable transgenics	5.2-kb (mouse)	*LacZ*	ECs of large- and medium-sized blood vessels in several tissues	Brain
	Hprt locus targeting	1.6-kb (human)	*LacZ*	ECs in arteries>veins	Cardiomyocytes, VSMC
EPCR	Stable transgenics	−1080 to −1, −350 to −1 (mouse)	GFP	Expression in multiple tissues of adult, although cell type not defined	
Flt-1	*Hprt* locus targeting	1-kb (human)	*LacZ*	ECs in all vascular beds except for the liver	Cardiomyocytes
ICAM-2	Stable transgenics	0.33- and 3-kb (human)	CD59	Uniform EC expression in both lines	Granulocytes
KDR/Flk-1	Transient and stable transgenics	Various promoter fragments	*LacZ*	939-bp promoter+510-bp first intron fragment drives expression in most ECs, but expression lost with age	
	Transient transgenics	−640 to +299 and 510-bp intronic enhancer; with or without mutation of HoxB5 binding site (mouse)	*LacZ*	Wild-type promoter directs uniform expression in ECs of embryo; mutation abolishes expression	
	Stable transgenics	4.5-kb+510-bp intronic enhancer (mouse)	Luciferase	Most tissues; decreased expression with age	
Mef2c	Transient transgenics	Various fragments (mouse)	*LacZ*	4.66-kb directs uniform EC expression in embryos; patchy expression in adult; mutation of Ets sites disrupted expression	Some circulating blood cells
Notch4	Transient transgenics	Various promoter fragments (human)	*LacZ*	Different promoter fragments, different vascular beds	
PAI-1	Stable transgenics	2.9-kb (human)	eGFP	2.9-kb directs expression in "glomeruli" and "venous structures" of lung	Kidney tubular cells; lung epithelial cells; pyramidal cells of cerebellum
Pre- and pro-endothelin	Stable transgenics	5.9-kb (murine)	Luc	ECs of arteries and arterioles>veins, capillaries	VSCM, certain epithelial cells
PrP	Stable transgenics	6.9-kb (bovine)	GFP	ECs of mucosal capillaries and venules in lamina propria mucosa of intestinal villi; small vessels of intestinal submucosa; peri-tubular vessels in renal cortex	Purkinje cells, lymphocytes, keratinocytes
Scl/tal-1	Transient transgenics	Various promoter fragments (mouse)	*LacZ*	5.5-kb directs expression to embryonic endothelium	Blood cells
Tie-1	Standard transgenics	0.74-kb (mouse)	*LacZ*	ECs in arterial segments at vascular junctions	
	Standard transgenics	1.15-kb (mouse)	Cre		
	Transient transgenics	0.54-kb (mouse) (WT and oct mutant)	*LacZ*	Patchy EC expression in embryo; reduced expression and cell-type specificity with oct mutant	

Table 2. continued

Gene	Assay	Promoter (species)	Reporter gene	Pattern of expression	Non-EC
Tie-1	Standard transgenics	0.8-kb (mouse)	*LacZ*	Predominantly ECs of arterioles and capillaries in adult mice	
		1.1-kb (mouse)	eGFP	Throughout vasculature of embryo up to E11.5; later in embryogenesis A>V; very little expression in adult vascular beds	
	Transient transgenics	Deletants and mutants of the 0.8-kb mouse promoter	*LacZ*	Ets mutations resulted in decreased EC expression in most organs	
	Standard transgenics	0.8-kb (mouse)	Growth hormone		
Tie-2	Standard transgenics	1.2-kb promoter (mouse)	*LacZ*	Patchy EC expression in embryo, absent in adult	
	Standard and transient transgenics	2.1-kb promoter+ 10-kb intron 1; several mutations also tested (mouse)	*LacZ*	Uniform EC expression of full-length WT promoter in embryo and adult	
		2.1-kb promoter+ 1.7-kb intronic enhancer (mouse)	t-TA	In binary system (Tie-2–tTA+TRE–β-galactosidase), "near uniform" expression in endothelium	
		2.1-kb promoter+10-kb intron 1 (mouse)	Cre	Bred with CAG-CAT-Z transgenic mice; in contrast to standard transgenic mice, expression in mesenchymal cells of atrioventricular canal (only embryos analyzed)	
	Hprt locus targeting	0.72-kb fragment containing a 300-bp intronic enhancer coupled upstream to a 423-bp core promoter—with or without Ets mutations (mouse)	*LacZ*	Widespread EC expression in adult (lost with Ets mutation, except in brain)	
VE-cadherin	Standard transgenics	−2486 to +24 (mouse)	CAT	ECs of all vascular beds except brain capillaries in adult	
vWF	Standard transgenics	−487 to +246 (human)	*LacZ*	ECs in blood vessels in adult brain	
	Standard transgenics	−2186 to end of first intron (human)	*LacZ*	ECs in blood vessels in brain, microvessels of heart and skeletal muscle in adult	
	Standard transgenics	−2645 and the end of the first intron (mouse)	*LacZ*	ECs in blood vessels in brain, microvessels of heart and skeletal muscle in adult; megakaryocytes in one line	
	Hprt locus targeting	−2186 to end of first intron (human)	*LacZ*	ECs in blood vessels in brain, microvessels of heart and skeletal muscle in adult	

See online supplement for references.
EC indicates endothelial cell; eNOS, endothelial nitric oxide synthase; EPCR, endothelial protein C receptor; GFP, green fluorescent protein; ICAM, intercellular adhesion molecule; PAI-1, plasminogen activator inhititor-1; PrP, prion protein; VSMC, vascular smooth muscle cells; vWF, von Willebrand factor.

other EC promoters were subsequently tested in standard transgenic mice (Table 2). Interestingly, the majority of these DNA sequences were found to direct vascular bed- or cell subtype-specific expression.

In standard transgenic mice, multiple copies of the promoter–reporter gene cassette are randomly integrated into the mouse genome (Figure 4). Random integration of variable transgene copies may give rise to significant line-to-line variation in expression. One approach to overcome these variables is to employ a "plug-in-socket" strategy in which a single copy of the transgene is inserted into a defined locus of the genome. For example, a number of EC-specific promoters (linked to the *LacZ* reporter gene) have been targeted to the *Hprt* locus of mice, using homologous recombination. Under these more stringent conditions, different EC promoters continue to direct expression to specific

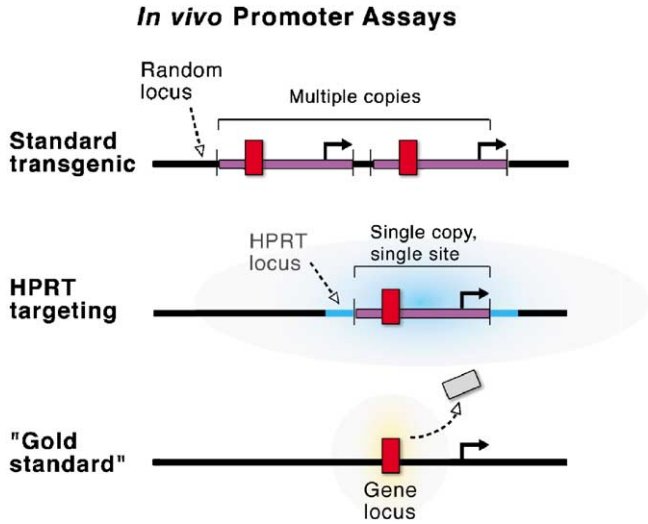

Figure 4. *In vivo assays for studying promoter function*. Three different approaches are shown. Standard transgenesis involves the injection of linearized DNA (containing promoter coupled to reporter gene cDNA) into fertilized oocytes. The procedure typically results in random integration of multiple transgene copies. In *HPRT* targeting, a single copy of the transgenic cassette is introduced into a defined (albeit heterologous) locus of the mouse genome through homologous recombination. In the last approach ("gold standard"), homologous recombination is employed to replace the endogenous wild-type allele with one containing a mutation of a single *cis*-regulatory element.

vascular beds (Evans et al. 2000, Guillot et al. 2000, Minami et al. 2002, 2003).

Thus, these various DNA promoter fragments represent molecular markers or probes for vascular bed-specific transcriptional activity. An important question is whether these differences reflect intrinsic (fixed) properties of the EC or whether they reflect differences within the cell's microenvironment. To cite a specific example, a fragment of the vWF promoter is expressed in blood vessels of the brain and the microvascular endothelium of the heart and skeletal muscle, but not in other organs (Aird et al. 1997). Do ECs in the brain, heart, and skeletal muscle possess cell-type-specific transcriptional networks that are unique to these cells? Or do these cells only appear different because they are following instructions that are unique to the tissue microenvironment? When noncardiac ECs of the mice were exposed to heart-derived signals, they expressed the transgene (Aird et al. 1997). In another study, the human Flt-1 and vWF promoters were targeted to the *Hprt* locus (Minami et al. 2002). The Flt-1 transgene was active in the endothelium of tumor xenografts, whereas the VWF promoter was not (Minami et al. 2002). Under in vitro conditions, conditioned medium from tumor cells resulted in a significant upregulation of Flt-1 mRNA and promoter activity, but no change in vWF levels (Minami et al. 2002). Taken together, these findings suggest that vascular bed specificity is programmed by the tissue microenvironment (at least in the case of vWF and Flt-1) and is not a fixed property of the EC.

One limitation that is common to standard transgenic and *Hprt*-targeting approaches is that exogenous DNA is introduced into a foreign genomic locus. Although the data from these studies provide powerful proof of principle for vascular bed-specific gene regulation, ultimate proof for the involvement of one or another *cis*-regulatory element (or DNA promoter fragment) in mediating vascular bed- or cell-subtype-specific regulation of a given gene will require selective deletion or mutation of that element from the endogenous locus (Figure 4). Such an approach has been carried out with other lineage-specific genes, but to our knowledge has yet to be applied in the endothelial field.

Other approaches for studying EC gene regulation in vivo are less direct and provide only circumstantial data. For example, the immunolocalization of transcription factors—with or without putative target genes—in the endothelium in health or disease may yield insight into vascular-bed-specific regulatory programs (Table 3). Alternatively, the forced overexpression and/or inhibition/knockout of a transcription factor in vivo may provide clues about downstream target genes and cellular function.

- **Bridging In Vitro and In Vivo Assays**

There are advantages and disadvantages associated with in vitro and in vivo approaches for studying EC gene regulation. The major advantage of EC cultures is that they enable high throughput, detailed, and tightly controlled analyses. The chief disadvantage is that the cultured EC has been uncoupled from its native microenvironment and is therefore a "shadow of its former self." In vivo approaches have the distinct advantage that they enable investigators to study ECs in the context of their native microenvironment. Disadvantages include the difficulty in controlling experimental conditions, the costs and time associated with carrying out such studies, and the enormous complexity of in vivo biology.

An important challenge then is to improve the validity of in vitro assays, to simplify the nature of the in vivo analyses, and to use one approach to inform the other. There are several ways to improve the biological meaningfulness of in vitro assays. First, to facilitate comparisons across studies, it would be helpful to qualify results according to the origin (species and vascular bed) and passage number of the cells. Second, it would be of interest to systematically compare transcriptional profiles in cultured (multi-passaged) ECs from different vascular beds. This approach, which has been carried out for some subtypes of cells (Chi et al. 2003, Lacorre et al. 2004), will capture those differences in gene expression that are intrinsic to the cells—that is, the site-specific properties that are epigenetically fixed and mitotically heritable. Finally, an important priority is to learn how to recapitulate the microenvironment in vitro. The many reports that describe the

Table 3. Examples of in situ localization of transcription factors in endothelium

Transcription factor	*Tissue*	*Pattern of expression*	*Assay*
ATF-3	Human iliac artery	ECs in atherosclerotic lesions	IH
c-Jun, NF-κB	Dog heart	Nuclear localization in venular endothelium in heart following brief ischemia	IH
Egr-1	Human carotid artery	Expression in ECs overlying atherosclerotic lesion	IH
Egr-1	Mouse organs	Vascular-bed-specific expression; site-specific induction with systemic administration of VEGF, EGF	IF
ERβ1, ERβcx/β2	Human endometrial tissue	Human endometrial ECs	IH
Ets-1	Human synovium	Synovial ECs in patients with RA	IH, ISH
HIF-1α, HIF-2α	Rat kidney	Various patterns of upregulation in ECs of renal cortex and medulla in response to ischemia, carbon monoxide, and cobaltous chloride	IH
HIF-1α and 1β	Human lung	Plexiform lesions in PH-PPH	IH, ISH
HoxD10, HoxD3	Human breast	HoxD10 in microvascular EC normal tissue, HoxD3 in neovessels of cancerous tissue	ISH
Id1 and Id3	Human synovium	RA synovial EC	IH
KLF2	Human thoracic aorta	Microheterogeneity in ECs	ISH
NF-AT	Human heart	Human pulmonary valve ECs	IH
NF-κB	Rat heart	ECs in infarcted heart	p65 IH
NF-κB	Rat aorta	ECs in aortas from rats with hyper- homocysteinemia	IF
NF-κB	Mouse aorta	Microheterogeneity in ECs; site-specific increases in endotoxemic and atherosclerosis models	IF
Nur77	Human coronary artery	ECs in atherosclerotic plaque	IH
Smad6/7	Human coronary artery	Smad 6 increased, Smad7 decreased in EC of atherosclerotic vessels	IH
SREBP-1	Pig aorta	Decreased in abdominal aorta of hypercholesterolemic pigs	mRNA in whole tissue (aortic rings)
SREBP	Rabbit skin	ECs in rabbit skin partial-thickness wound-healing model (neovessels)	IF
STAT-5	Human carotid artery	ECs in atherosclerotic lesions	IF

See online supplement for references.
ATF indicates activating transcription factor; EC, endothelial cell; EGF, epidermal growth factor; ER, estrogen receptor; HIF, hypoxia inducible factor; HOX, homeobox; Id, inhibitor of DNA binding; IF, immunofluorescence; IH, immunohistochemistry; ISH, in situ hybridization; KLF, Kruppel-like factor; NF-AT, nuclear factor of activated T cell; RA, rheumatoid arthritis; SREBP, sterol regulatory element binding protein; STAT, signal transducer and activator of transcription; VEGF, vascular endothelial growth factor.

effect of one or another agonist on gene regulation in ECs represent an important starting point. Goals for the future include studying the effects of multiple mediators added at different concentrations and in different time sequences. Moreover, the continued development of co-culture systems (and neutralizing antibodies to soluble mediators) should provide new insights into environmentally regulated gene expression.

From the standpoint of in vivo assays, important goals are to elucidate the distribution of transcription factors in the endothelium and to develop a catalogue of vascular bed-specific EC promoters that direct expression of a gene-of-interest to one or another vascular bed. One of the best ways to understand a biological system is to place it under stress and observe its response. For example, the use of mouse or rat models of sepsis has yielded fascinating results that point to the highly complex and vascular bed-specific response capacity of the endothelium. Increasingly, the study of EC properties in disease states (e.g., cancer, pulmonary hypertension, and rheumatoid arthritis) is providing clues to basic mechanisms of gene regulation. Endothelial-cell-specific knockout of transcription factors promises to improve our understanding of transcriptional networks in this cell type. Finally, future improvements in the resolution of laser capture microdissection will provide an invaluable platform for mapping the transcriptome in intact endothelium.

- **Revisiting the Definition of an Endothelial Cell**

The endothelium consists of a giant mosaic of phenotypes. Indeed, it seems likely that at any given point in time, there do not exist two phenotypically identical ECs in the human body. Thus, with the exception of its anatomic location, there are few unifying features of the endothelium.

Perhaps an EC is defined less by any panel of cell-type-specific mRNA or protein markers, and more by its *behavioral repertoire* (Figure 5). With each EC representing an input–output device, the endothelium is analogous to a biosensor or barcode reader, constantly taking inventory of its immediate microenvironment. The EC transcriptional machinery serves to integrate and transduce these extracellular signals, generating an output that is appropriate for that time and place (see

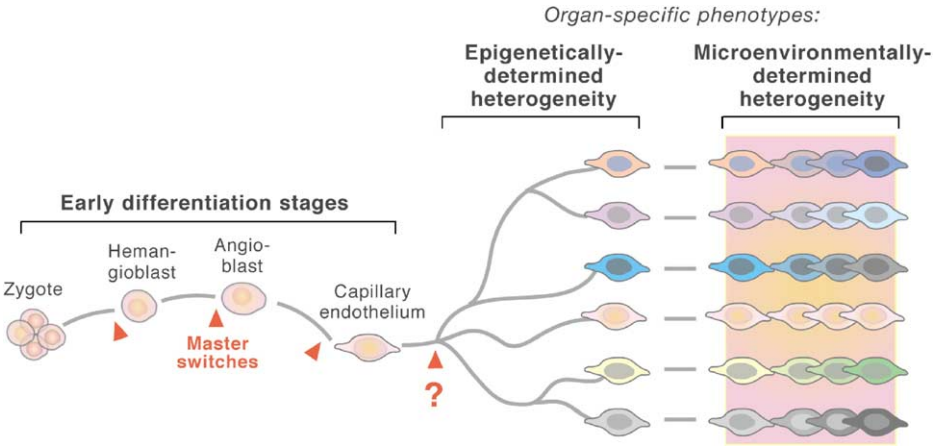

Figure 5. *The behavioral repertoire of the endothelium*. Endothelial-cell phenotypes arise from two mechanisms. First, epigenetic modification of DNA leads to mitotically heritable changes (these are shown as "master switches", or triangles). Data support the role for such switches in mediating early lineage determination and perhaps arterial/venous identity (the latter is not shown). The extent to which epigenetic events normally dictate organ or site-specific phenotypes of microvascular endothelium is not clear (shown as question mark, and labeled as "epigenetically determined heterogeneity"). In contrast, there is increasing evidence that many site-specific properties of capillary endothelium are reversibly governed by signals residing in the microenvironment ("microenvironmentally determined heterogeneity"). Each color shade represents a distinct cellular phenotype (e.g., transcriptome, proteome, and/or function).

model, Figure 6). Like all cells of the human body, ECs follow local rules to generate complex global behavior. The uniqueness of the endothelium lies not so much in its constituent parts (e.g., cells), but rather in its emergent properties of plasticity, adaptability, and heterogeneity.

• Unanswered Questions

Previous studies of EC gene regulation have provided important insights into vascular biology. However, many questions still remain. For example, how do non-cell-type-specific transcription factors cooperate to promote EC-restricted expression? Are there as of yet undiscovered "master switches," which, like MyoD in skeletal muscle or PU.1 in the myeloid lineage, govern lineage determination and/or expression of cell-type-specific genes in the adult endothelium? If and when bone-marrow-derived ECs repopulate the intact vasculature, to what extent do these cells acquire the phenotype of the resident ECs? Is disease and/or aging associated with increased epigenetic changes within the endothelium?[1] Do the promoter regions of EC genes contain functionally relevant polymorphisms? The answer to these, and many other interesting questions related to EC gene regulation, requires further study.

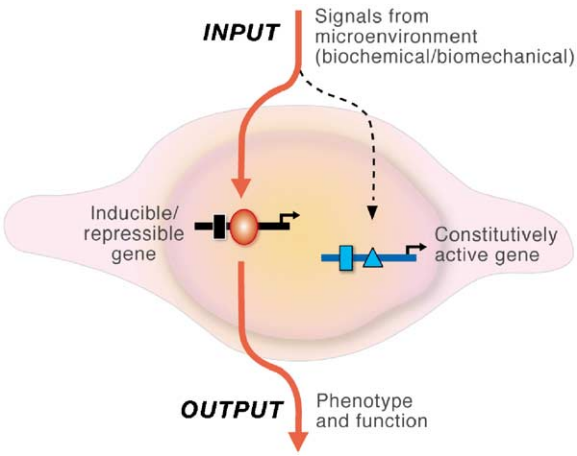

Figure 6. *Model of endothelial gene regulation*. Consistent with the analogy of the EC as an input–output device, many genes are modulated by signaling pathways, which begin in the extracellular milieu and end at distinct regions (or modules) of the gene promoter. Inducible/repressible and constitutively expressed genes are normally regulated by non-cell-type-specific transcription factors. The dotted line indicates that many, if not all, of so-called constitutive genes are coupled (at least to some extent) to the extracellular environment. The black and blue lines represent promoters of modulatable and constitutively expressed genes, respectively. The arrows represent transcriptional start sites, and the shapes denote *cis*-regulatory elements.

• Acknowledgments

This work was supported in part by the National Institute of Health Grants HL076540 and HL36028. Artwork was done by Steven Moskowitz.

• Appendix A. Abbreviations for Table 2

Agonist abbreviations: ABCA1, ATP-binding cassette transporter-1; AGE, advanced glycation end products; Ang-1, angiopoietin-1; Ang II, angiotensin II, ANP, atrial natriuretic peptide; CRP, C reactive protein; EBV, Epstein-Barr virus; EPCR, endothelial protein C receptor; ET, endothelin; FGF, fibroblast growth factor; GM-CSF, granulocyte monocyte-colony stimulating factor; Hb, hemoglobin; HGF, hepatocyte growth factor; HHV, human herpesvirus; HIV, human immunodeficiency virus; HMGB1, High mobility group box 1; HMG-Co, hydroxymethylglutaryl-coen-

[1] Preliminary studies point to the importance of aging and disease in mediating epigenetic changes in endothelium. For example, in co-culture studies, cardiac myocytes induce the expression of PDGF-B in passaged cardiac microvascular ECs isolated from 3-month-old mice, but not 18-month-old C57BL/6 mice (Edelberg et al. 2002). Endothelial cells cultured (passage 3–6) from the aorta of diabetic mice (db/db) display increased monocyte adhesion, compared with ECs cultured from wild-type C57BL/6 mice (Hatley et al. 2003).

zyme A; IGF, insulin like growth factor; IFN, interferon; IL, interleukin; LDL, low density lipoprotein; LIX, lipopolysaccharide-induced CXC chemokine; LMP, latent membrane protein; LPL, lipoprotein lipase; NO, nitric oxide; PAF, platelet activating factor; PMP, polymorphic membrane protein; TGF, transforming growth factor; TNF, tumor necrosis factor; t-PA, tissue type plasminogen activator; VEGF, vascular endothelial growth factor; VLDL, very low density lipoprotein.

Transcription factor abbreviations: AP-1, activating protein-1; ATF, Activating transcription factor; CREB, cyclic AMP response element binding protein; dbpb, DNA-binding protein B; Egr-1, early growth response-1; GR, glucocorticoids receptor; HIF, hypoxia inducible factor; Id, inhibitor of DNA binding; IRF, interferon regulatory factor; KLF, Kruppel-like factor; LXR, liver X receptor; NF-AT, nuclear factor of activated T cell; NF-1, nuclear factor-1; PPAR, peroxisome proliferator-activated receptor; RUNX, Runt-related gene; SREBP, sterol regulatory element binding protein; STAT, signal transducer and activator of transcription; Tcf, T-cell-specific factor.

Target gene abbreviations: ACE, angiotensin converting enzyme; COX, cyclo-oxygenase; CRLR, calcitonin receptor-like receptor; DAF, decay accelerating factor; DSCR, Down syndrome critical region; eNOS, endothelial nitric oxide synthase; ET, endothelin; HO-1, hemeoxygenase-1; ICAM, intercellular adhesion molecule; iNOS, inducible nitric oxide synthase; IRF1, interferon regulatory factor-1; MCP, monocyte chemoattractant protein; MDR, multiple drug resistance; MMP, matrix metalloproteinase; MnSOD, manganese superoxide dismutase; NQO1, NAD(P)H:quinone oxidoreductase-1; PAI, plasminogen activator inhibitor; PDGF, platelet-derived growth factor; RANKL, receptor activator of nuclear factor kappa B ligand; SDF, stromal cell-derived factor-1; SOCS3, suppressor of cytokine signaling 3; TAP-1, transporter associated with antigen processing-1; TF, tissue factor; u-PA, urokinase type plasminogen activator; VCAM, vascular cellular adhesion molecule.

Cell abbreviations: bAdEC, bovine adrenal cortex capillary EC; BLEC, bovine lung microvascular EC; BPAEC, bovine pulmonary artery EC; BREC, bovine retinal microvascular EC; CDEC, mouse cardiac-derived EC; GenC, mouse glomerular EC; HAEC, human aortic EC; HBME-1, human bone marrow EC; HBMVEC, human brain microvascular EC; HCMEC, human cardiac microvascular EC; HMEC-1, human microvascular EC line; HMVEC, human microvascular EC; HPMEC, human pulmonary microvascular EC; HPVEC, human pulmonary valve EC; HSVEC, human saphenous vein EC; MaoEC, murine aortic EC; MBEC, murine cerebral EC; PAEC, porcine aortic EC; PRET, porcine retinal EC; RCEC, rat cardiac EC; RPAEC, rat pulmonary aortic EC; RPVEC, rat pulmonary microvascular EC.

• Appendix B. References for Tables

Table 1. Examples of agonist-mediated induction/repression of transcription factors and downstream target genes in EC †

Agonist	Transcription factor	Target genes/cell function	Cell type(s)	Ref
Oxygenation				
Hypoxia	AP-1	HO-1	RPAEC	1
	AP-1	eNOS	PAEC	2
	Egr-1	——-	BAEC	3
	Elk-1	——-	BAEC	3
	HIF	CRLR	HMEC	4
	HIF	Endoglin	HMEC-1	5
	HIF	MDR-1	BAEC	6
	HIF	SDF-1	HUVEC	7
	HIF+GATA-2+AP-1	ET-1	HUVEC	8
	NERF-2	TIE-2	HUVEC	9
	Smad2/3	TGF-β	HUVEC	10,11
	Sp1	MDR-1	BAEC	6
Hemodynamic forces				
Laminar Shear Stress	GATA-6	Inhibits u-PA	HCAEC	12
	KLF2	——-	HUVEC	13
	Negative SSRE binding protein	Inhibits Tie-1	BAEC	14
	c-Myc	MMP-9	Murine lymphoid EC line	15
	NF-κB	ICAM-1	BAEC	16
	NF-κB	eNOS	BAEC	17
	Inhibits NF-κB	Inhibits PDGF-B	BAEC	18
	GR	——-	BAEC	19

Table 1 continued

Agonist	Transcription factor	Target genes/cell function	Cell type(s)	Ref
Hemodynamic forces				
Laminar Shear Stress	Sp1	Flk-1	HUVEC	20,21
	Inhibits Sp1	Inhibits P2X4	HUVEC, BAEC	22
	SREBP	——-	BAEC	23,24
	AP-1	eNOS	Lamb fetal vs. pulmonary artery EC	25
	Nrf2	NQO1, HO-1	HAEC, HMEC	26
	CRE binding protein	COX-2	BAEC	27
Cyclical strain	AP-1	ET-1	HUVEC	28
Stretch	NF-κB	IL-6	HUVEC	29
Chemokines, and Cytokines				
IFN-α	STAT-4	MCP-1, SOCS3	HUVEC	30
IFN-γ	STAT-1	CD40	HUVEC	31
	STAT-1	TAP-1, IRF1, Class 1 MHC	HUVEC	32
IL-1	AP-1, Sp1	ICAM-1	mAoEC	33
	NF-κB, C/EBP	iNOS	rPVEC	34
IL-4	STAT-1	MCP-1	HUVEC	35
	AP-2, NF-1, Sp1, STAT-6, but not NF-κB or AP-1	——-	HUVEC	36
IL-6	STAT-3	——-	BAEC	37
IL-10	STAT-3	eNOS	HUVEC	38
IL-18	NF-κB	IL-1, TNF-α	HCMEC	39
LIX	NF-κB	IL-1, TNF-α	rCEC	40
PAF	STAT-3	——-	HUVEC	41,42
TNF-α	ATF3	Cell death	HUVEC	43
	CREB	——-	HUVEC	44
	E2F1 inhibition	Inhibits E2F1	BAEC	45
	Id1, Id3	E-selectin, not ICAM-1	HUVEC	46
	IRF-1	VCAM-1	HAEC	47
	NF-κB	E-selectin, TF	HDMEC vs. HUVEC	48
	NF-κB	Fractalkine	HUVEC	49
	NF-κB	IL-8	HAEC	50
	NF-κB	MadCAM	SVEC	51
	NF-κB	MCP-1, IL-8, ICAM-1, VCAM-1, E-selectin	HUVEC	52
	NF-κB	TF	HUVEC	53
	NUR77	PAI-1	HUVEC	54
	Sp1	Fractalkine	HUVEC	49
	Sp1, Sp3 inhibition	Inhibits eNOS	BAEC	55
TNF-α + IFN-γ	STAT-1, IRF	CD40	HUVEC	31
HMGB1	NF-κB, Sp1	——-	HMEC-1	56
Growth factors, hormones, glucose				
AGE	NF-κB, AP-1	VEGF	HDMEC	57
Ang-1	Forkhead	——-	HUVEC, BLMEC	58
Dihydrotestosterone	Androgen receptor/NF-kB	VCAM-1	HUVEC, BAEC	59
ET-1	Ets-1	——-	BAEC	60
FGF-2	Tcf-4	Cyclin D1	HUVEC	61
	STAT-3	——-	HUVEC	42
	Egr-1		BAEC	62
Glucose	AP-1	Fibronectin	HUVEC, HMEC	63
	AP-1	IL-8	HAEC, PAEC	64
	Egr-1	Flt-1, PAI-1	GEnC	65
	NF-κB	VCAM-1	HAEC	66
	NF-κB	eNOS, inhibits cell migration	HAEC	67
	NF-κB	Fibronectin	HUVEC, HMEC	63

Table 1 continued

Agonist	Transcription factor	Target genes/cell function	Cell type(s)	Ref
GM-CSF	NF-κB	——-	HUVEC	68
HGF	Forkhead	——-	HCAEC	69
	Ets-1	MMP-1, HGF, c-met	HAEC	70
	Egr-1	ACE	BPAEC	71
IGF-1	RUNX2	Tube formation	HBME-1	72,73
Insulin	AP-1	eNOS	PAEC	74
	Egr-1	Flt-1, PAI-1	GEnC	65
Leptin	AP-1	ET-1	HUVEC, BAEC	75
	STAT-3	VEGF	PRET	76
Placental growth factor	AP-1	——-	HUVEC, HPAEC	77
Prolactin (16-kDa N' terminal fragment)	NF-κB	Caspases 3, 8, 9; E-selectin	bAdEC	78
Relaxin	NF-κB	ET_B	HUVEC, BAEC	79
VEGF	Ets-1	Neuropilin, Angiopoietin-2, cell proliferation	BREC	80
	Ets-1	Angiopoietin-2	HUVEC	81
	NF-AT	DSCR-1	HUVEC	82
	NF-AT	Proliferation	HPVEC	83
	NF-AT	DAF	HUVEC	84
	GATA	DSCR-1	HUVEC	82
	GATA	COX-2	HMEC-1	85
	Forkhead	$p27^{kip1}$	HCAEC	86
	Forkhead	MnSOD	HCAEC	69
	NF-κB	MnSOD	HCAEC	69
	NF-κB	TF	HUVEC	87
	STAT-3	VEGF	BREC, BAEC, HDMEC	88
	Egr-1	TF, Flt-1, u-PA		89
	Id1, Id3	ICAM-1, but not E-selectin; EC proliferation	HUVEC	46
	SREBP1/2	——-	HPMEC	90
	Sp1	KDR	BAEC	91
	Vezf1	VEGF-mediated proliferation, migration and tube formation	MSS31 mouse EC line	92
Thymosin β4	AP-1	PAI-1	EA.hy 926	93
Lipoproteins and related mediators				
Cholesterol	AP-1	ICAM-1	HUVEC	94
Diabetic LDL	STAT-5B	p21 and G1 fraction	HUVEC	95
Native LDL	LXR	ABCA1	HUVEC, HCAEC	96
oxHDL	NF-κB	——	HUVEC	97
oxLDL	ATF3	Cell death	HUVEC	43
VLDL, oxVLDL	CREB, NF-κB	——-	Eahy926, HUVEC	98
LPL+VLDL	PPARα	Ac-CoA-oxidase	BAEC	99
LPA, S-1-P	NF-κB	ICAM-1	HUVEC	100
Fatty acids	Sp1	Heparanase	HMVEC, BAEC	101
Oleic acid	NF-κB	ET-1	HAEC	102
5(S)-HETE	STAT-3	——-	HMVEC	103
Oxidized phospholipids (OxPAPC)	STAT-3	IL-8	HAEC	104
	NFAT, Egr-1	TF	HUVEC	53
	CREB	HO-1	HUVEC	105
	Egr-1	——-	HUVEC	106
Epoxyeicosatrienoic acids (EET)	CRE	t-PA	HSVEC	107
Linoleic acid	NF-κB, AP-1	——-	HUVEC	108
Omega-3 fatty acids	PPAR-α	——-	HUVEC, BAEC	109
Matrix and related factors				

Table 1 continued

Agonist	Transcription factor	Target genes/cell function	Cell type(s)	Ref
Fibronectin	NF-κB	E-selectin, VCAM-1, ET-1, CYR-61	HUVEC	110
3-dimensional collagen type-1	GATA-2	MMP-2	Rat skeletal muscle EC	111
Type 1 collagen	Ets-1	MMP-1	BAEC	60
TGF-β	Smad2	eNOS	BAEC, HUVEC, BAEC	112
	Smad1/2/5, Id	Cell migration	BAEC, mouse embryonic EC	113
	CREB	RANKL	Bone marrow-derived EC line	114
	Smad3/4, AP-1	ET-1	HUVEC, BAEC	115
	Smad6	Inhibits TM	HUVEC	116
Serine proteases				
Thrombin	AP-1	Prepro-ET	PAEC	117
	dbpB	PDGF-B	HUVEC, BAEC	118
	dbpB	EPCR	BAEC	119
	Egr-1	——-	HPAEC	120
	NF-κB	ICAM-1	HUVEC	121
	NF-κB, GATA-2	VCAM-1	HUVEC	122
	NF-AT	DSCR-1	HUVEC	82
	Sp1	KDR	BAEC	91
	SRF	Egr-1	HPAEC	120
Cell-cell interactions				
Platelet-EC	NF-κB	MCP-1, VCAM-1		123
Neutrophil-EC	Ets-1	Tube formation	BAEC	124
Neutrophil-derived proteases	NF-κB (cleaves and inhibits)		HUVEC	125
Vasomotor tone				
NO	Inhibits NF-κB	Inhibits ET-1	PAEC	126
	Nrf2	HO-1	BAEC	127
Ang II	AP-1	ET-1	HUVEC	128
	NF-κB	VCAM-1, ICAM-1	HUVEC, BAEC	129
ANP	AP-1	HO-1	HUVEC	130
Pathogens				
Orienta tsutsugamushi	AP-1, NF-κB	MCP-1	HUVEC	131
Chlamydia pneumoniae PMP	NF-κB	IL-6, MCP-1		
	HUVEC	132		
Chlamydia pneumoniae infection	NF-κB	ICAM-1	HAEC	133
Shiga toxin	NF-κB	MCP-1, IL-8	HUVEC/GEC	134
African swine fever virus (infection)	Decreases p65 NF-κB	Inhibits IL-8, E-selectin, but increases TF	PAEC, bushpig EC	135
Bartonella henselae (infection)	NF-κB	ICAM-1, E-selectin	HUVEC	136
Bartonella bacilliformis infection	AP-1	——-	HUVEC	137
EBV infection or LMP-1 over-expression	NF-κB	——-	HUVEC	138
HIV protein Tat	NF-κB	E-selectin	HUVEC	139
HIV pcTat	NF-κB	Monocyte adhesion	HUVEC	140
HIV Tat1-72	NF-κB, AP-1	——-	Porcine brain MEC	141
HHV-8 (retroviral infection of ORF74)	NF-κB	——-	HDMEC	142
Drugs/toxins				
Arsenite	NF-κB, AP-1	——-	PAEC	143
Bleomycin	Egr-1	ACE	BPAEC	144
	NF-κB	E-selectin	HUVEC	145

Table 1 continued

Agonist	Transcription factor	Target genes/cell function	Cell type(s)	Ref
	NF-κB, Nrf-1/2	γ-glutamylcysteine synthetase	BPAEC, rat pulmonary microvascular EC	146
Cocaine	NF-κB, AP-1	——-	HBMVEC	147
Doxorubicin	p53, not NF-κB	CD95	HUVEC	148
	NF-κB	Cell apoptosis	BAEC	149
HMG-Co reductase inhibitors	Inhibits NF-κB, AP-1, HIF-1α	——-	Ea.hy926, HAEC	150
Isoproterenol	NF-κB	IL-18	CDEC	151
Methamphetamine	NF-κB, AP-1	TNF-α	hBMVEC	152
Disease-related mediators				
Human lymphoma-derived soluble factors	NF-κB	Leukocyte adhesion	HUVEC	153
Amyloid-beta peptide	AP-1	Bim	mBEC	154
Anti-phospholipid antibodies	NF-κB	——-	HUVEC, HMEC-1	155
Homocysteine	NF-κB	Fas	HUVEC	156
	NF-κB	——-	HUVEC, HAEC	157
MetHb	NF-κB	E-selectin, IL-6, IL-8	HUVEC	158

Table 2. Promoter Analysis in transgenic mice

Gene	Assay	Promoter	Reporter gene	Pattern of expression	Non-EC	Ref
Activin Receptor-like Kinase 1	Stable transgenics	Various promoter fragments (mouse)	LacZ	9.2-kb promoter directs arterial specific expression in adults		159
CD34	Stable transgenics	160-kb (human)	Human CD34	EC in many vascular beds in adult	Bone marrow	160
c-mpl	Stable transgenics	2-kb (mouse)	Human placental alkaline phosphatase	Some EC in blood vessels of embryo (and yolk sac); none in adult	Megakaryocytes	161
eNOS	Stable transgenics	1.6-kb bp (human)	LacZ	Expression in EC of brain, heart, skeletal muscle, and aorta. β- galactosidase activity was consistently absent in vascular beds of the liver, kidney, and spleen		162
	Stable transgenics	5.2-kb (mouse)	LacZ	EC of large and medium sized blood vessels in several tissues	Brain	163
	Hprt locus targeting	1.6-kb (human)	LacZ	EC in arteries > veins	Cardiomyocytes, VSMC	164
EPCR	Stable transgenics	-1080 to –1, -350 to –1 (mouse)	GFP	Expression in multiple tissues of adult, though cell type not defined		119
Flt-1	Hprt locus targeting	1-kb (human)	LacZ	EC in all vascular beds except for the liver	Cardiomyocytes	165
ICAM-2	Stable transgenics	0.33-kb and 3-kb (human)	CD59	Uniform EC expression in both lines	Granulocytes	166
KDR/Flk-1	Transient and stable transgenics	Various promoter fragments	LacZ	939-bp promoter + 510-bp first intron fragment drives expression in most EC, but expression lost with age		167
	Transient transgenics	-640 - + 299 and 510-bp intronic enhancer; with or without mutation of HoxB5 binding site (mouse)	LacZ	Wild type promoter directs uniform expression in EC of embryo; mutation abolishes expression		168

Table 2 continued

Gene	Assay	Promoter	Reporter gene	Pattern of expression	Non-EC	Ref
	Stable transgenics	4.5 Kb + 510-bp intronic enhancer (mouse)	Luciferase	Most tissues; Decreased expression with age		169
Mef2c	Transient transgenics	Various fragments (mouse)	LacZ	4.66-kb directs uniform EC expression in embryos; patchy expression in adult; mutation of Ets sites disrupted expression	Some circulating blood cells	170
Notch4	Transient transgenics	Various promoter fragments (human)	LacZ	Different promoter fragments, different vascular beds		171
PAI-1	Stable transgenics	2.9-kb (human)	eGFP	2.9-kb directs expression in "glomeruli" and "venous structures" of lung	Kidney tubular cells; lung epithelial cells; pyramidal cells of cerebellum	172
Pre-pro-endothelin	Stable transgenics	5.9-kb (murine)	Luc	EC of arteries and arterioles > veins, capillaries	VSCM, certain epithelial cells	173
PrP	Stable transgenics	6.9-kb (bovine)	GFP	EC of mucosal capillaries and venules in lamina propria mucosa of intestinal villi; small vessels of intestinal submucosa; peri-tubular vessels in renal cortex	Purkinje cells, lymphocytes, keratinocytes	174
SCL/TAL-1	Transient transgenics	Various promoter fragments (mouse)	LacZ	5.5-kb directs expression to embryonic endothelium	Blood cells	175
TIE-1	Standard transgenics	0.74-kb (mouse)	LacZ	EC in arterial segments at vascular junctions		176
	Standard transgenics	1.15-kb (mouse)	Cre			177
	Transient transgenics	0.54-kb (mouse) (WT and oct mutant)	LacZ	Patchy EC expression in embryo; reduced expression and cell type specificity with Oct mutant		178
	Standard transgenics	0.8-kb (mouse)	LacZ	Predominantly EC of arterioles and capillaries in adult mice		179
		1.1 kb (mouse)	eGFP	Throughout vasculature of embryo up to E11.5; later in embryogenesis A>V; very little expression in adult vascular beds		180
	Transient transgenics	Deletants and mutants of the 0.8 kb mouse promoter	LacZ	Ets mutations resulted in decreased EC expression in most organs		181
	Standard transgenics	0.8 kb (mouse)	Growth hormone			181
TIE-2	Standard transgenics	1.2 kb promoter (mouse)	LacZ	Patchy EC expression in embryo, absent in adult		182
	Standard and transient transgenics	2.1 kb promoter + 10 kb intron 1; several mutations also tested (mouse)	LacZ	Uniform EC expression of full length WT promoter in embryo and adult		183
		2.1 kb promoter + 1.7 kb intronic enhancer (mouse)	t-TA	In binary system (Tie-2-tTA + TRE-βGal), "near uniform" expression in endothelium		184
		2.1 kb promoter + 10 kb intron 1 (mouse)	Cre	Bred with CAG-CAT-Z transgenic mice; In contrast to standard transgenic mice, expression in mesenchymal cells of atrioventricular canal (only embryos analyzed)		185
	Hprt locus targeting	0.72-kb fragment containing a 300-bp intronic enhancer coupled upstream to a 423-bp core promoter – with or without Ets mutations (mouse)	LacZ	Widespread EC expression in adult (lost with Ets mutation, except in brain)		186

Table 2 continued

Gene	Assay	Promoter	Reporter gene	Pattern of expression	Non-EC	Ref
VE-cadherin	Standard transgenics	-2486 to +24 (mouse)	CAT	EC of all vascular beds except brain capillaries in adult		187
vWF	Standard transgenics	-487-+246 (human)	LacZ	EC in blood vessels in adult brain		188
	Standard transgenics	-2186 – to end of first intron (human)	LacZ	EC in blood vessels in brain, microvessels of heart and skeletal muscle in adult		189
	Standard transgenics	-2645 and the end of the first intron (mouse)	LacZ	EC in blood vessels in brain, microvessels of heart and skeletal muscle in adult; megakaryocytes in one line		190
	Hprt locus targeting	-2186 – to end of first intron (human)	LacZ	EC in blood vessels in brain, microvessels of heart and skeletal muscle in adult		165

Table 3. Examples of in situ localization of transcription factors in endothelium

Transcription factor	Tissue	Pattern of Expression	Assay	Ref
ATF-3	Human iliac artery	EC of atherosclerotic lesions	IH	43
c-Jun, NF-κB	Dog heart	Nuclear localization in venular endothelium in heart following brief ischemia	IH	191
Egr-1	Human carotid artery	Expression in EC overlying atherosclerotic lesion	IH	192
Egr-1	Mouse organs	Vascular bed specific expression; site-specific induction with systemic administration of VEGF, EGF	IF	193
ERβ1, ERβcx/β2	Human endometrial tissue	Human endometrial EC	IH	194
Ets-1	Human synovium	Synovial EC in patients with RA	IH, ISH	195
HIF-1α, HIF-2α	Rat kidney	Various patterns of upregulation EC of renal cortex and medulla in response to ischemia, carbon monoxide, and cobaltous chloride	IH	196
HIF-1α and 1β	Human lung	Plexiform lesions in PH-PPH	IH, ISH	197
HoxD10, HoxD3	Human breast	HoxD10 in microvascular EC normal tissue, HoxD3 in neovessels of cancerous tissue	ISH	198
Id1 and Id3	Human synovium	RA synovial EC	IH	199
KLF2	Human thoracic aorta	Microheterogeneity in EC	ISH	200
NF-AT	Human heart	Human pulmonary valve EC	IH	83
NF-κB	Rat heart	EC in infarcted heart	p65 IH	201
NF-κB	Rat aorta	EC in aortas from rats with hyper- homocysteinemia	IF	157
NF-κB	Mouse aorta	Microheterogeneity in EC; site-specific increases in endotoxemic and atherosclerosis models	IF	202
Nur77	Human coronary artery	EC in atherosclerotic plaque	IH	54
Smad6/7	Human coronary artery	Smad 6 increased, Smad7 decreased in EC of atherosclerotic vessels	IH	116
SREBP-1	Pig aorta	Decreased in abdominal aorta of hypercholesterolemic pigs	mRNA in whole tissue (aortic rings)	203
SREBP	Rabbit skin	EC in rabbit skin partial-thickness wound-healing model (neovessels)	IF	90
STAT-5	Human carotid artery	EC of atherosclerotic lesions	IF	95

1. Ryter, S.W. et al. (2002) Mitogen activated protein kinase (MAPK) pathway regulates heme oxygenase-1 gene expression by hypoxia in vascular cells. Antioxid Redox Signal 4 (4), 587-592

2. Hoffmann, A. et al. (2001) Hypoxia-induced upregulation of eNOS gene expression is redox-sensitive: a comparison between hypoxia and inhibitors of cell metabolism. J Cell Physiol 188 (1), 33-44

3. Lo, L.W. et al. (2001) Endothelial exposure to hypoxia induces Egr-1 expression involving PKCalpha-mediated Ras/Raf-1/ERK1/2 pathway. J Cell Physiol 188 (3), 304-312.

4. Nikitenko, L.L. et al. (2003) Transcriptional regulation of the CRLR gene in human microvascular endothelial cells by hypoxia. Faseb J 17 (11), 1499-1501

5. Sanchez-Elsner, T. et al. (2002) Endoglin expression is regulated by transcriptional cooperation between the hypoxia and transforming growth factor-beta pathways. J Biol Chem 277 (46), 43799-43808

6. Comerford, K.M. et al. (2002) Hypoxia-inducible factor-1-dependent regulation of the multidrug resistance (MDR1) gene. Cancer Res 62 (12), 3387-3394

7. Ceradini, D.J. et al. (2004) Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 10 (8), 858-864

8. Yamashita, K. et al. (2001) Molecular regulation of the endothelin-1 gene by hypoxia. Contributions of hypoxia-inducible factor-1, activator protein-1, GATA-2, AND p300/CBP. J Biol Chem 276 (16), 12645-12653.

9. Christensen, R.A. et al. (2002) NERF2, a member of the Ets family of transcription factors, is increased in response to hypoxia and angiopoietin-1: a potential mechanism for Tie2 regulation during hypoxia. J Cell Biochem 85 (3), 505-515

10. Zhang, H. et al. (2003) Cellular response to hypoxia involves signaling via Smad proteins. Blood 101 (6), 2253-2260

11. Akman, H.O. et al. (2001) Response to hypoxia involves transforming growth factor-beta2 and Smad proteins in human endothelial cells. Blood 98 (12), 3324-3331

12. Sokabe, T. et al. (2004) Differential regulation of urokinase-type plasminogen activator expression by fluid shear stress in human coronary artery endothelial cells. Am J Physiol Heart Circ Physiol 287 (5), H2027-2034

13. SenBanerjee, S. et al. (2004) KLF2 Is a novel transcriptional regulator of endothelial proinflammatory activation. J Exp Med 199 (10), 1305-1315

14. Chen-Konak, L. et al. (2003) Transcriptional and post-translation regulation of the Tie1 receptor by fluid shear stress changes in vascular endothelial cells. Faseb J 17 (14), 2121-2123

15. Magid, R. et al. (2003) Expression of matrix metalloproteinase-9 in endothelial cells is differentially regulated by shear stress. Role of c-Myc. J Biol Chem 278 (35), 32994-32999

16. Tzima, E. et al. (2002) Activation of Rac1 by shear stress in endothelial cells mediates both cytoskeletal reorganization and effects on gene expression. Embo J 21 (24), 6791-6800

17. Davis, M.E. et al. (2004) Shear stress regulates endothelial nitric-oxide synthase promoter activity through nuclear factor kappaB binding. J Biol Chem 279 (1), 163-168

18. Eng, E. and Ballermann, B.J. (2003) Diminished NF-kappaB activation and PDGF-B expression in glomerular endothelial cells subjected to chronic shear stress. Microvasc Res 65 (3), 137-144

19. Ji, J.Y. et al. (2003) Shear stress causes nuclear localization of endothelial glucocorticoid receptor and expression from the GRE promoter. Circ Res 92 (3), 279-285

20. Urbich, C. et al. (2003) Fluid shear stress-induced transcriptional activation of the vascular endothelial growth factor receptor-2 gene requires Sp1-dependent DNA binding. FEBS Lett 535 (1-3), 87-93

21. Abumiya, T. et al. (2002) Shear stress induces expression of vascular endothelial growth factor receptor Flk-1/KDR through the CT-rich Sp1 binding site. Arterioscler Thromb Vasc Biol 22 (6), 907-913

22. Korenaga, R. et al. (2001) Sp1-mediated downregulation of P2X4 receptor gene transcription in endothelial cells exposed to shear stress. Am J Physiol Heart Circ Physiol 280 (5), H2214-2221

23. Lin, T. et al. (2003) Rho-ROCK-LIMK-cofilin pathway regulates shear stress activation of sterol regulatory element binding proteins. Circ Res 92 (12), 1296-1304

24. Liu, Y. et al. (2002) Shear stress activation of SREBP1 in endothelial cells is mediated by integrins. Arterioscler Thromb Vasc Biol 22 (1), 76-81

25. Wedgwood, S. et al. (2003) Developmental differences in the shear stress-induced expression of endothelial NO synthase: changing role of AP-1. Am J Physiol Lung Cell Mol Physiol 284 (4), L650-662

26. Chen, X.L. et al. (2003) Laminar flow induction of antioxidant response element-mediated genes in endothelial cells. A novel anti-inflammatory mechanism. J Biol Chem 278 (2), 703-711

27. Inoue, H. et al. (2002) Transcriptional and posttranscriptional regulation of cyclooxygenase-2 expression by fluid shear stress in vascular endothelial cells. Arterioscler Thromb Vasc Biol 22 (9), 1415-1420

28. Cheng, T.H. et al. (2001) Reactive oxygen species mediate cyclic strain-induced endothelin-1 gene expression via Ras/Raf/extracellular signal-regulated kinase pathway in endothelial cells. J Mol Cell Cardiol 33 (10), 1805-1814

29. Kobayashi, S. et al. (2003) Stretch-induced IL-6 secretion from endothelial cells requires NF-kappaB activation. Biochem Biophys Res Commun 308 (2), 306-312

30. Torpey, N. et al. (2004) Interferon alpha but not interleukin 12 activates STAT4. signaling in human vascular endothelial cells. J Biol Chem 279 (25), 26789-26796

31. Wagner, A.H. et al. (2002) Cytokine-inducible CD40 expression in human endothelial cells is mediated by interferon regulatory factor-1. Blood 99 (2), 520-525

32. Mahboubi, K. and Pober, J.S. (2002) Activation of signal transducer and activator of transcription 1 (STAT1) is not sufficient for the induction of STAT1-dependent genes in endothelial cells. Comparison of interferon-gamma and oncostatin M. J Biol Chem 277 (10), 8012-8021

33. Berendji-Grun, D. et al. (2001) Nitric oxide inhibits endothelial IL-1[beta]-induced ICAM-1 gene expression at the transcriptional level decreasing Sp1 and AP-1 activity. Mol Med 7 (11), 748-754

34. Kolyada, A.Y. and Madias, N.E. (2001) Transcriptional regulation of the human iNOS gene by IL-1beta in endothelial cells. Mol Med 7 (5), 329-343

35. Lee, Y.W. et al. (2003) Redox-regulated mechanisms of IL-4-induced MCP-1 expression in human vascular endothelial cells. Am J Physiol Heart Circ Physiol 284 (1), H185-192

36. Lee, Y.W. et al. (2001) Interleukin 4 induces transcription of the 15-lipoxygenase I gene in human endothelial cells. J Lipid Res 42 (5), 783-791

37. Ni, C.W. et al. (2004) Interleukin-6-induced JAK2/STAT3 signaling pathway in endothelial cells is suppressed by hemodynamic flow. Am J Physiol Cell Physiol 287 (3), C771-780

38. Cattaruzza, M. et al. (2003) Interleukin-10 induction of nitric-oxide synthase expression attenuates CD40-mediated interleukin-12 synthesis in human endothelial cells. J Biol Chem 278 (39), 37874-37880

39. Chandrasekar, B. et al. (2004) Activation of intrinsic and extrinsic proapoptotic signaling pathways in interleukin-18-mediated human cardiac endothelial cell death. J Biol Chem 279 (19), 20221-20233

40. Chandrasekar, B. et al. (2003) Chemokine-cytokine cross-talk. The ELR+ CXC chemokine LIX (CXCL5) amplifies a proinflammatory cytokine response via a phosphatidylinositol 3-kinase-NF-kappa B pathway. J Biol Chem 278 (7), 4675-4686

41. Deo, D.D. et al. (2004) Activation of platelet-activating factor receptor-coupled G alpha q leads to stimulation of Src and focal adhesion kinase via two separate pathways in human umbilical vein endothelial cells. J Biol Chem 279 (5), 3497-3508

42. Deo, D.D. et al. (2002) Phosphorylation of STAT-3 in response to basic fibroblast growth factor occurs through a mechanism involving platelet-activating factor, JAK-2, and Src in human umbilical vein endothelial cells. Evidence for a dual kinase mechanism. J Biol Chem 277 (24), 21237-21245

43. Nawa, T. et al. (2002) Expression of transcriptional repressor ATF3/LRF1 in human atherosclerosis: colocalization and possible involvement in cell death of vascular endothelial cells. Atherosclerosis 161 (2), 281-291

44. Gustin, J.A. et al. (2004) Tumor necrosis factor activates CRE-binding protein through a p38 MAPK/MSK1 signaling pathway in endothelial cells. Am J Physiol Cell Physiol 286 (3), C547-555

45. Kishore, R. et al. (2003) Tumor necrosis factor-mediated E2F1 suppression in endothelial cells: differential requirement of c-Jun N-terminal kinase and p38 mitogen-activated protein kinase signal transduction pathways. Circ Res 93 (10), 932-940

46. Sakurai, D. et al. (2004) Crucial role of inhibitor of DNA binding/differentiation in the vascular endothelial growth factor-induced activation and angiogenic processes of human endothelial cells. J Immunol 173 (9), 5801-5809

47. Dagia, N.M. et al. (2004) Phenyl methimazole inhibits TNF-alpha-induced VCAM-1 expression in an IFN regulatory factor-1-dependent manner and reduces monocytic cell adhesion to endothelial cells. J Immunol 173 (3), 2041-2049

48. O'Reilly, F.M. et al. (2003) Regulation of tissue factor in microvascular dermal endothelial cells. J Invest Dermatol 120 (3), 489-494

49. Ahn, S.Y. et al. (2004) Tumor necrosis factor-alpha induces fractalkine expression preferentially in arterial endothelial cells and mithramycin A suppresses TNF-alpha-induced fractalkine expression. Am J Pathol 164 (5), 1663-1672

50. Yeh, M. et al. (2001) Increased transcription of IL-8 in endothelial cells is differentially regulated by TNF-alpha and oxidized phospholipids. Arterioscler Thromb Vasc Biol 21 (10), 1585-1591

51. Oshima, T. et al. (2001) Regulation and distribution of MAdCAM-1 in endothelial cells in vitro. Am J Physiol Cell Physiol 281 (4), C1096-1105

52. Denk, A. et al. (2001) Activation of NF-kB via the IkB kinase complex is both essential and sufficient for proinflammatory gene expression in primary endothelial cells. J Biol Chem 3, 3

53. Bochkov, V.N. et al. (2002) Oxidized phospholipids stimulate tissue factor expression in human endothelial cells via activation of ERK/EGR-1 and Ca(++)/NFAT. Blood 99 (1), 199-206

54. Gruber, F. et al. (2003) Direct binding of Nur77/NAK-1 to the plasminogen activator inhibitor 1 (PAI-1) promoter regulates TNF alpha -induced PAI-1 expression. Blood 101 (8), 3042-3048

55. Anderson, H.D. et al. (2004) Tumor necrosis factor-alpha inhibits endothelial nitric-oxide synthase gene promoter activity in bovine aortic endothelial cells. J Biol Chem 279 (2), 963-969

56. Fiuza, C. et al. (2003) Inflammation-promoting activity of HMGB1 on human microvascular endothelial cells. Blood 101 (7), 2652-2660

57. Okamoto, T. et al. (2002) Angiogenesis induced by advanced glycation end products and its prevention by cerivastatin. Faseb J 16 (14), 1928-1930

58 Daly, C. et al. (2004) Angiopoietin-1 modulates endothelial cell function and gene expression via the transcription factor FKHR (FOXO1). Genes Dev 18 (9), 1060-1071

59. Death, A.K. et al. (2004) Dihydrotestosterone promotes vascular cell adhesion molecule-1 expression in male human endothelial cells via a nuclear factor-kappaB-dependent pathway. Endocrinology 145 (4), 1889-1897

60. Naito, S. et al. (2002) Ets-1 upregulates matrix metalloproteinase-1 expression through extracellular matrix adhesion in vascular endothelial cells. Biochem Biophys Res Commun 291 (1), 130-138

61. Holnthoner, W. et al. (2002) Fibroblast growth factor-2 induces Lef/Tcf-dependent transcription in human endothelial cells. J Biol Chem 277 (48), 45847-45853

62. Santiago, F.S. et al. (1999) Early growth response factor-1 induction by injury is triggered by release and paracrine activation by fibroblast growth factor-2. Am J Pathol 154 (3), 937-944.

63. Chen, S. et al. (2003) High glucose-induced, endothelin-dependent fibronectin synthesis is mediated via NF-kappa B and AP-1. Am J Physiol Cell Physiol 284 (2), C263-272

64. Srinivasan, S. et al. (2003) Glucose regulates monocyte adhesion through endothelial production of interleukin-8. Circ Res 92 (4), 371-377

65. Hasan, R.N. et al. (2003) Differential regulation of early growth response gene-1 expression by insulin and glucose in vascular endothelial cells. Arterioscler Thromb Vasc Biol 23 (6), 988-993

66. Kouroedov, A. et al. (2004) Selective inhibition of protein kinase Cbeta2 prevents acute effects of high glucose on vascular cell adhesion molecule-1 expression in human endothelial cells. Circulation 110 (1), 91-96

67. Hamuro, M. et al. (2002) High glucose induced nuclear factor kappa B mediated inhibition of endothelial

cell migration. Atherosclerosis 162 (2), 277-287

68. Ebner, K. et al. (2003) GMCSF activates NF-kappaB via direct interaction of the GMCSF receptor with IkappaB kinase beta. Blood 102 (1), 192-199

69. Abid, M.R. et al. (2004) Vascular endothelial growth factor-mediated induction of manganese superoxide dismutase occurs through redox-dependent regulation of forkhead and IkappaB/NF-kappaB. J Biol Chem 279 (42), 44030-44038

70. Tomita, N. et al. (2003) Angiogenic property of hepatocyte growth factor is dependent on upregulation of essential transcription factor for angiogenesis, ets-1. Circulation 107 (10), 1411-1417

71. Day, R.M. et al. (2004) Hepatocyte growth factor regulates angiotensin converting enzyme expression. J Biol Chem 279 (10), 8792-8801

72. Qiao, M. et al. (2004) Insulin-like growth factor-1 regulates endogenous RUNX2 activity in endothelial cells through a phosphatidylinositol 3-kinase/ERK-dependent and Akt-independent signaling pathway. J Biol Chem 279 (41), 42709-42718

73. Sun, L. et al. (2001) Runt-related gene 2 in endothelial cells: inducible expression and specific regulation of cell migration and invasion. Cancer Res 61 (13), 4994-5001.

74. Fisslthaler, B. et al. (2003) Insulin enhances the expression of the endothelial nitric oxide synthase in native endothelial cells: a dual role for Akt and AP-1. Nitric Oxide 8 (4), 253-261

75. Quehenberger, P. et al. (2002) Leptin induces endothelin-1 in endothelial cells in vitro. Circ Res 90 (6), 711-718

76. Suganami, E. et al. (2004) Leptin stimulates ischemia-induced retinal neovascularization: possible role of vascular endothelial growth factor expressed in retinal endothelial cells. Diabetes 53 (9), 2443-2448

77. Holmes, D.I. and Zachary, I. (2004) Placental growth factor induces FosB and c-Fos gene expression via Flt-1 receptors. FEBS Lett 557 (1-3), 93-98

78. Tabruyn, S.P. et al. (2003) The antiangiogenic factor 16K human prolactin induces caspase-dependent apoptosis by a mechanism that requires activation of nuclear factor-kappaB. Mol Endocrinol 17 (9), 1815-1823

79. Dschietzig, T. et al. (2003) Relaxin, a pregnancy hormone, is a functional endothelin-1 antagonist: attenuation of endothelin-1-mediated vasoconstriction by stimulation of endothelin type-B receptor expression via ERK-1/2 and nuclear factor-kappaB. Circ Res 92 (1), 32-40

80. Watanabe, D. et al. (2004) Transcription factor Ets-1 mediates ischemia- and vascular endothelial growth factor-dependent retinal neovascularization. Am J Pathol 164 (5), 1827-1835

81. Hasegawa, Y. et al. (2004) Transcriptional regulation of human angiopoietin-2 by transcription factor Ets-1. Biochem Biophys Res Commun 316 (1), 52-58

82. Minami, T. et al. (2004) Vascular endothelial growth factor- and thrombin-induced termination factor, down syndrome critical region-1, attenuates endothelial cell proliferation and angiogenesis. J Biol Chem 279 (48), 50537-50554

83. Johnson, E.N. et al. (2003) NFATc1 mediates vascular endothelial growth factor-induced proliferation of human pulmonary valve endothelial cells. J Biol Chem 278 (3), 1686-1692.

84. Mason, J.C. et al. (2004) Decay-accelerating factor induction on vascular endothelium by vascular endothelial growth factor (VEGF) is mediated via a VEGF receptor-2 (VEGF-R2)- and protein kinase C-alpha/epsilon (PKCalpha/epsilon)-dependent cytoprotective signaling pathway and is inhibited by cyclosporin A. J Biol Chem 279 (40), 41611-41618

85. Tamura, M. et al. (2002) Vascular endothelial growth factor up-regulates cyclooxygenase-2 expression in human endothelial cells. J Clin Endocrinol Metab 87 (7), 3504-3507

86. Abid, M.R. et al. (2004) Vascular endothelial growth factor activates PI3K/Akt/forkhead signaling in endothelial cells. Arterioscler Thromb Vasc Biol 24 (2), 294-300

87. Kim, I. et al. (2002) Angiopoietin-1 negatively regulates expression and activity of tissue factor in endothelial cells. Faseb J 16 (1), 126-128.

88. Bartoli, M. et al. (2003) VEGF differentially activates STAT3 in microvascular endothelial cells. Faseb J 17 (11), 1562-1564

89. Lucerna, M. et al. (2003) NAB2, a corepressor of EGR-1, inhibits vascular endothelial growth factor-mediated gene induction and angiogenic responses of endothelial cells. J Biol Chem 278 (13), 11433-11440

90. Zhou, R.H. et al. (2004) Vascular endothelial growth factor activation of sterol regulatory element binding protein: a potential role in angiogenesis. Circ Res 95 (5), 471-478

91. Wang, J. et al. (2002) Induction of KDR expression in bovine arterial endothelial cells by thrombin: involvement of nitric oxide. J Cell Physiol 190 (2), 238-250

92. Miyashita, H. et al. (2004) Vascular endothelial zinc finger 1 is involved in the regulation of angiogenesis: possible contribution of stathmin/OP18 as a downstream target gene. Arterioscler Thromb Vasc Biol 24 (5), 878-884

93. Al-Nedawi, K.N. et al. (2004) Thymosin beta 4 induces the synthesis of plasminogen activator inhibitor 1 in cultured endothelial cells and increases its extracellular expression. Blood 103 (4), 1319-1324

94. Yuan, Y. et al. (2001) Cholesterol enrichment upregulates intercellular adhesion molecule-1 in human vascular endothelial cells. Biochim Biophys Acta 1534 (2-3), 139-148

95. Brizzi, M.F. et al. (2002) Diabetic LDL inhibits cell-cycle progression via STAT5B and p21(waf). J Clin Invest 109 (1), 111-119

96. Liao, H. et al. (2002) Native LDL upregulation of ATP-binding cassette transporter-1 in human vascular endothelial cells. Arterioscler Thromb Vasc Biol 22 (1), 127-132

97. Matsunaga, T. et al. (2003) NF-kappa B activation in endothelial cells treated with oxidized high-density lipoprotein. Biochem Biophys Res Commun 303 (1), 313-319

98. Norata, G.D. et al. (2003) Gene expression and intracellular pathways involved in endothelial dysfunction induced by VLDL and oxidised VLDL. Cardiovasc Res 59 (1), 169-180

99. Ziouzenkova, O. et al. (2003) Lipolysis of triglyceride-rich lipoproteins generates PPAR ligands: evidence for an antiinflammatory role for lipoprotein lipase. Proc Natl Acad Sci U S A 100 (5), 2730-2735

100. Lee, H. et al. (2004) Lysophospholipids increase ICAM-1 expression in HUVEC through a Gi- and NF-kappaB-dependent mechanism. Am J Physiol Cell Physiol 287 (6), C1657-1666

101. Chen, G. et al. (2004) Inflammatory cytokines and fatty acids regulate endothelial cell heparanase expression. Biochemistry 43 (17), 4971-4977

102. Park, J.Y. et al. (2003) Oleic acid induces endothelin-1 expression through activation of protein kinase C and NF-kappa B. Biochem Biophys Res Commun 303 (3), 891-895

103. Zeng, Z.Z. et al. (2002) 5(S)-hydroxyeicosatetraenoic acid stimulates DNA synthesis in human microvascular endothelial cells via activation of Jak/STAT and phosphatidylinositol 3-kinase/Akt signaling, leading to induction of expression of basic fibroblast growth factor 2. J Biol Chem 277 (43), 41213-41219

104. Yeh, M. et al. (2004) Oxidized phospholipids increase interleukin 8 (IL-8) synthesis by activation of the c-src/signal transducers and activators of transcription (STAT)3 pathway. J Biol Chem 279 (29), 30175-30181

105. Kronke, G. et al. (2003) Oxidized phospholipids induce expression of human heme oxygenase-1 involving activation of cAMP-responsive element-binding protein. J Biol Chem 278 (51), 51006-51014

106. Kadl, A. et al. (2002) Analysis of inflammatory gene induction by oxidized phospholipids in vivo by quantitative real-time RT-PCR in comparison with effects of LPS. Vascul Pharmacol 38 (4), 219-227

107. Node, K. et al. (2001) Activation of Galpha s mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoic acids. J Biol Chem 276 (19), 15983-15989

108. Toborek, M. et al. (2002) Unsaturated fatty acids selectively induce an inflammatory environment in human endothelial cells. Am J Clin Nutr 75 (1), 119-125

109. Sethi, S. et al. (2002) Oxidized omega-3 fatty acids in fish oil inhibit leukocyte-endothelial interactions through activation of PPAR alpha. Blood 100 (4), 1340-1346

110. Klein, S. et al. (2002) Alpha 5 beta 1 integrin activates an NF-kappa B-dependent program of gene expression important for angiogenesis and inflammation. Mol Cell Biol 22 (16), 5912-5922

111. Han, X. et al. (2003) Transcriptional up-regulation of endothelial cell matrix metalloproteinase-2 in response to extracellular cues involves GATA-2. J Biol Chem 278 (48), 47785-47791

112. Saura, M. et al. (2002) Smad2 mediates transforming growth factor-beta induction of endothelial nitric oxide synthase expression. Circ Res 91 (9), 806-813

113. Goumans, M.J. et al. (2002) Balancing the activation state of the endothelium via two distinct TGF-beta type I receptors. Embo J 21 (7), 1743-1753

114. Ishida, A. et al. (2002) Transforming growth factor-beta induces expression of receptor activator of NF-kappa B ligand in vascular endothelial cells derived from bone. J Biol Chem 277 (29), 26217-26224

115. Rodriguez-Pascual, F. et al. (2003) Functional cooperation between Smad proteins and activator protein-1 regulates transforming growth factor-beta-mediated induction of endothelin-1 expression. Circ Res 92 (12), 1288-1295

116. Sandusky, G. et al. (2002) Modulation of thrombomodulin-dependent activation of human protein C through differential expression of endothelial Smads. J Biol Chem 277 (51), 49815-49819

117. Kitazumi, K. and Tasaka, K. (1993) The role of c-Jun protein in thrombin-stimulated expression of pre-proendothelin-1 mRNA in porcine aortic endothelial cells. Biochem Pharmacol 46 (3), 455-464.

118. Stenina, O.I. et al. (2000) Thrombin activates a Y box-binding protein (DNA-binding protein B) in endothelial cells. J Clin Invest 106 (4), 579-587.

119. Gu, J.M. et al. (2000) Characterization and regulation of the 5'-flanking region of the murine endothelial protein C receptor gene. J Biol Chem 275 (17), 12481-12488.

120. Wu, S.Q. et al. (2002) The proximal serum response element in the Egr-1 promoter mediates response to thrombin in primary human endothelial cells. Blood 100 (13), 4454-4461

121. Rahman, A. et al. (1999) Thrombin-induced p65 homodimer binding to downstream NF-kappa B site of the promoter mediates endothelial ICAM-1 expression and neutrophil adhesion. J Immunol 162 (9), 5466-5476.

122. Minami, T. and Aird, W.C. (2001) Thrombin stimulation of the vascular cell adhesion molecule-1 promoter in endothelial cells is mediated by tandem nuclear factor-kappa B and GATA motifs. J Biol Chem 276 (50), 47632-47641.

123. Xu, J.W. et al. (2004) Upregulation of endothelial nitric oxide synthase by cyanidin-3-glucoside, a typical anthocyanin pigment. Hypertension 44 (2), 217-222

124. Yasuda, M. et al. (2002) Differential roles of ICAM-1 and E-selectin in polymorphonuclear leukocyte-induced angiogenesis. Am J Physiol Cell Physiol 282 (4), C917-925

125. Preston, G.A. et al. (2002) Novel effects of neutrophil-derived proteinase 3 and elastase on the vascular endothelium involve in vivo cleavage of NF-kappaB and proapoptotic changes in JNK, ERK, and p38 MAPK signaling pathways. J Am Soc Nephrol 13 (12), 2840-2849

126. Ohkita, M. et al. (2003) The role of nuclear factor-kappa B in the regulation of endothelin-1 production by nitric oxide. Eur J Pharmacol 472 (3), 159-164

127. Buckley, B.J. et al. (2003) Nitric oxide stimulates Nrf2 nuclear translocation in vascular endothelium. Biochem Biophys Res Commun 307 (4), 973-979

128. Hsu, Y.H. et al. (2004) Role of reactive oxygen species-sensitive extracellular signal-regulated kinase pathway in angiotensin II-induced endothelin-1 gene expression in vascular endothelial cells. J Vasc Res 41 (1), 64-74

129. Costanzo, A. et al. (2003) Endothelial activation by angiotensin II through NFkappaB and p38 pathways: Involvement of NFkappaB-inducible kinase (NIK), free oxygen radicals, and selective inhibition by aspirin. J Cell Physiol 195 (3), 402-410

130. Kiemer, A.K. et al. (2003) Characterization of heme oxygenase 1 (heat shock protein 32) induction by atrial natriuretic peptide in human endothelial cells. Endocrinology 144 (3), 802-812

131. Cho, N.H. et al. (2002) Induction of the gene encoding macrophage chemoattractant protein 1 by Orientia tsutsugamushi in human endothelial cells involves activation of transcription factor activator protein 1. Infect Immun 70 (9), 4841-4850

132. Niessner, A. et al. (2003) Polymorphic membrane protein (PMP) 20 and PMP 21 of Chlamydia pneumoniae induce proinflammatory mediators in human endothelial cells in vitro by activation of the nuclear factor-kappaB pathway. J Infect Dis 188 (1), 108-113

133. Vielma, S.A. et al. (2003) Chlamydophila pneumoniae induces ICAM-1

expression in human aortic endothelial cells via protein kinase C-dependent activation of nuclear factor-kappaB. Circ Res 92 (10), 1130-1137

134. Zoja, C. et al. (2002) Shiga toxin-2 triggers endothelial leukocyte adhesion and transmigration via NF-kappaB dependent up-regulation of IL-8 and MCP-1. Kidney Int 62 (3), 846-856

135. Vallee, I. et al. (2001) African swine fever virus infection of porcine aortic endothelial cells leads to inhibition of inflammatory responses, activation of the thrombotic state, and apoptosis. J Virol 75 (21), 10372-10382

136. Fuhrmann, O. et al. (2001) Bartonella henselae induces NF-kappaB-dependent upregulation of adhesion molecules in cultured human endothelial cells: possible role of outer membrane proteins as pathogenic factors. Infect Immun 69 (8), 5088-5097

137. Verma, A. and Ihler, G.M. (2002) Activation of Rac, Cdc42 and other downstream signalling molecules by Bartonella bacilliformis during entry into human endothelial cells. Cell Microbiol 4 (9), 557-569

138. Xiong, A. et al. (2004) Epstein-Barr virus latent membrane protein 1 activates nuclear factor-kappa B in human endothelial cells and inhibits apoptosis. Transplantation 78 (1), 41-49

139. Cota-Gomez, A. et al. (2002) The human immunodeficiency virus-1 Tat protein activates human umbilical vein endothelial cell E-selectin expression via an NF-kappa B-dependent mechanism. J Biol Chem 277 (17), 14390-14399

140. Pieper, G.M. et al. (2002) Transfection of human endothelial cells with HIV-1 tat gene activates NF-kappa B and enhances monocyte adhesion. Am J Physiol Heart Circ Physiol 283 (6), H2315-2321

141. Toborek, M. et al. (2003) HIV-Tat protein induces oxidative and inflammatory pathways in brain endothelium. J Neurochem 84 (1), 169-179

142. Pati, S. et al. (2001) Activation of NF-kappaB by the human herpesvirus 8 chemokine receptor ORF74: evidence for a paracrine model of Kaposi's sarcoma pathogenesis. J Virol 75 (18), 8660-8673

143. Tsou, T.C. et al. (2003) The protective role of NF-kappaB and AP-1 in arsenite-induced apoptosis in aortic endothelial cells. Toxicol Appl Pharmacol 191 (2), 177-187

144. Day, R.M. et al. (2001) Bleomycin upregulates gene expression of angiotensin-converting enzyme via mitogen-activated protein kinase and early growth response 1 transcription factor. Am J Respir Cell Mol Biol 25 (5), 613-619

145. Ishii, H. and Takada, K. (2002) Bleomycin induces E-selectin expression in cultured umbilical vein endothelial cells by increasing its mRNA levels through activation of NF-kappaB/Rel. Toxicol Appl Pharmacol 184 (2), 88-97

146. Day, R.M. et al. (2002) Bleomycin upregulates expression of gamma-glutamylcysteine synthetase in pulmonary artery endothelial cells. Am J Physiol Lung Cell Mol Physiol 282 (6), L1349-1357

147. Lee, Y.W. et al. (2001) Cocaine activates redox-regulated transcription factors and induces TNF-alpha expression in human brain endothelial cells. Brain Res 920 (1-2), 125-133

148. Lorenzo, E. et al. (2002) Doxorubicin induces apoptosis and CD95 gene expression in human primary endothelial cells through a p53-dependent mechanism. J Biol Chem 277 (13), 10883-10892

149. Wang, S. et al. (2002) Activation of nuclear factor-kappaB during doxorubicin-induced apoptosis in endothelial cells and myocytes is pro-apoptotic: the role of hydrogen peroxide. Biochem J 367 (Pt 3), 729-740

150. Dichtl, W. et al. (2003) HMG-CoA reductase inhibitors regulate inflammatory transcription factors in human endothelial and vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 23 (1), 58-63

151. Chandrasekar, B. et al. (2004) Beta-adrenergic stimulation induces interleukin-18 expression via beta2-AR, PI3K, Akt, IKK, and NF-kappaB. Biochem Biophys Res Commun 319 (2), 304-311

152. Lee, Y.W. et al. (2001) Methamphetamine induces AP-1 and NF-kappaB binding and transactivation in human brain endothelial cells. J Neurosci Res 66 (4), 583-591

153. Estrada-Bernal, A. et al. (2003) NF-kappaB dependent activation of human endothelial cells treated with soluble products derived from human lymphomas. Cancer Lett 191 (2), 239-248

154. Yin, K.J. et al. (2002) Amyloid-beta induces Smac release via AP-1/Bim activation in cerebral endothelial cells. J Neurosci 22 (22), 9764-9770

155. Raschi, E. et al. (2003) Role of the MyD88 transduction signaling pathway in endothelial activation by antiphospholipid antibodies. Blood 101 (9), 3495-3500

156. Suhara, T. et al. (2004) Homocysteine enhances endothelial apoptosis via upregulation of Fas-mediated pathways. Hypertension 43 (6), 1208-1213

157. Au-Yeung, K.K. et al. (2004) Hyperhomocysteinemia activates nuclear factor-kappaB in endothelial cells via oxidative stress. Circ Res 94 (1), 28-36

158. Liu, X. and Spolarics, Z. (2003) Methemoglobin is a potent activator of endothelial cells by stimulating IL-6 and IL-8 production and E-selectin membrane expression. Am J Physiol Cell Physiol 285 (5), C1036-1046

159. Seki, T. et al. (2004) Isolation of a regulatory region of activin receptor-like kinase 1 gene sufficient for arterial endothelium-specific expression. Circ Res 94 (8), e72-77

160. Radomska, H.S. et al. (2002) Transgenic targeting with regulatory elements of the human CD34 gene. Blood 100 (13), 4410-4419

161. Ziegler, S. et al. (2002) A 2-kb c-mpl promoter fragment is sufficient to direct expression to the megakaryocytic lineage and sites of embryonic hematopoiesis in transgenic mice. Blood 100 (3), 1072-1074.

162. Guillot, P.V. et al. (1999) A vascular bed-specific pathway. J Clin Invest 103 (6), 799-805.

163. Teichert, A.M. et al. (2000) In vivo expression profile of an endothelial nitric oxide synthase promoter-reporter transgene. Am J Physiol Heart Circ Physiol 278 (4), H1352-1361.

164. Guillot, P.V. et al. (2000) Targeting of human eNOS promoter to the Hprt locus of mice leads to tissue-restricted transgene expression. Physiol Genomics 2 (2), 77-83.

165. Minami, T. et al. (2002) Differential regulation of the von Willebrand factor and Flt-1 promoters in the endothelium of hypoxanthine phosphoribosyltransferase- targeted mice. Blood 100 (12), 4019-4025.

166. Cowan, P.J. et al. (1998) The human ICAM-2 promoter is endothelial cell-specific in vitro and in vivo and contains critical Sp1 and GATA binding sites. J Biol Chem 273 (19), 11737-11744.

167. Kappel, A. et al. (1999) Identification of vascular endothelial growth factor (VEGF) receptor-2 (Flk-1) promoter/enhancer sequences sufficient for angioblast and endothelial cell-specific transcription in transgenic mice. Blood 93 (12), 4284-4292.

168. Wu, Y. et al. (2003) HoxB5 is an upstream transcriptional switch for differentiation of the vascular endothelium from precursor cells. Mol Cell Biol 23 (16), 5680-5691

169. Zhang, N. et al. (2004) Tracking angiogenesis induced by skin wounding and contact hypersensitivity using a Vegfr2-luciferase transgenic mouse. Blood 103 (2), 617-626

170. De Val, S. et al. (2004) Mef2c is activated directly by Ets transcription factors through an evolutionarily conserved endothelial cell-specific enhancer. Dev Biol 275 (2), 424-434

171. Wu, J. et al. (2005) Molecular Determinants of NOTCH4 Transcription in Vascular Endothelium. Mol Cell Biol 25 (4), 1458-1474

172. Eren, M. et al. (2003) Tissue- and agonist-specific regulation of human and murine plasminogen activator inhibitor-1 promoters in transgenic mice. J Thromb Haemost 1 (11), 2389-2396

173. Harats, D. et al. (1995) Targeting gene expression to the vascular wall in transgenic mice using the murine preproendothelin-1 promoter. J Clin Invest 95 (3), 1335-1344.

174. Lemaire-Vieille, C. et al. (2000) Epithelial and endothelial expression of the green fluorescent protein reporter gene under the control of bovine prion protein (PrP) gene regulatory sequences in transgenic mice. Proc Natl Acad Sci U S A 97 (10), 5422-5427

175. Sanchez, M. et al. (1999) An SCL 3' enhancer targets developing endothelium together with embryonic and adult haematopoietic progenitors. Development 126 (17), 3891-3904

176. Porat, R.M. et al. (2004) Specific induction of tie1 promoter by disturbed flow in atherosclerosis-prone vascular niches and flow-obstructing pathologies. Circ Res 94 (3), 394-401

177. Gustafsson, E. et al. (2001) Tie-1-directed expression of Cre recombinase in endothelial cells of embryoid bodies and transgenic mice. J Cell Sci 114 (Pt 4), 671-676.

178. Boutet, S.C. et al. (2001) Identification of an octamer element required for in vivo expression of the TIE1 gene in endothelial cells. Biochem J 360 (Pt 1), 23-29

179. Korhonen, J. et al. (1995) Endothelial-specific gene expression directed by the tie gene promoter in vivo. Blood 86 (5), 1828-1835.

180. Iljin, K. et al. (2002) A fluorescent Tie1 reporter allows monitoring of vascular development and endothelial cell isolation from transgenic mouse embryos. Faseb J 16 (13), 1764-1774.

181. Iljin, K. et al. (1999) Role of ets factors in the activity and endothelial cell specificity of the mouse Tie gene promoter. Faseb J 13 (2), 377-386.

182. Schlaeger, T.M. et al. (1995) Vascular endothelial cell lineage-specific promoter in transgenic mice. Development 121 (4), 1089-1098.

183 Schlaeger, T.M. et al. (1997) Uniform vascular-endothelial-cell-specific gene expression in both embryonic and adult transgenic mice. Proc Natl Acad Sci U S A 94 (7), 3058-3063.

184. Teng, P.I. et al. (2002) Inducible and selective transgene expression in murine vascular endothelium. Physiol Genomics 11 (2), 99-107

185. Kisanuki, Y.Y. et al. (2001) Tie2-Cre transgenic mice: a new model for endothelial cell-lineage analysis in vivo. Dev Biol 230 (2), 230-242.

186. Minami, T. et al. (2003) Ets Motifs Are Necessary for Endothelial Cell-Specific Expression of a 723-bp Tie-2 Promoter/Enhancer in Hprt Targeted Transgenic Mice. Arterioscler Thromb Vasc Biol 23 (11), 2041-2047

187. Gory, S. et al. (1999) The vascular endothelial-cadherin promoter directs endothelial-specific expression in transgenic mice. Blood 93 (1), 184-192.

188. Aird, W.C. et al. (1995) Human von Willebrand factor gene sequences target expression to a subpopulation of endothelial cells in transgenic mice. Proc Natl Acad Sci U S A 92 (10), 4567-4571.

189. Aird, W.C. et al. (1997) Vascular bed-specific expression of an endothelial cell gene is programmed by the tissue microenvironment. J Cell Biol 138 (5), 1117-1124.

190. Guan, J. et al. (1999) Characterization of the mouse von Willebrand factor promoter. Blood 94 (10), 3405-3412.

191. Lakshminarayanan, V. et al. (2001) Reactive oxygen intermediates induce monocyte chemotactic protein-1 in vascular endothelium after brief ischemia. Am J Pathol 159 (4), 1301-1311

192. McCaffrey, T.A. et al. (2000) High-level expression of Egr-1 and Egr-1-inducible genes in mouse and human atherosclerosis. J Clin Invest 105 (5), 653-662.

193. Liu, L. et al. (2000) Egr-1 gene is induced by the systemic administration of the vascular endothelial growth factor and the epidermal growth factor. Blood 96 (5), 1772-1781.

194. Critchley, H.O. et al. (2002) Wild-type estrogen receptor (ERbeta1) and the splice variant (ERbetacx/beta2) are both expressed within the human endometrium throughout the normal menstrual cycle. J Clin Endocrinol Metab 87 (11), 5265-5273

195. Wernert, N. et al. (2002) The Ets 1 transcription factor is upregulated during inflammatory angiogenesis in rheumatoid arthritis. J Mol Med 80 (4), 258-266

196. Rosenberger, C. et al. (2002) Expression of hypoxia-inducible factor-1alpha and -2alpha in hypoxic and ischemic rat kidneys. J Am Soc Nephrol 13 (7), 1721-1732

197. Tuder, R.M. et al. (2001) Expression of angiogenesis-related molecules in plexiform lesions in severe pulmonary hypertension: evidence for a process of disordered angiogenesis. J Pathol 195 (3), 367-374

198. Myers, C. et al. (2002) Sustained expression of homeobox D10 inhibits angiogenesis. Am J Pathol 161 (6), 2099-2109

199. Sakurai, D. et al. (2001) Expression of ID family genes in the synovia from patients with rheumatoid arthritis. Biochem Biophys Res Commun 284 (2), 436-442

200. Dekker, R.J. et al. (2002) Prolonged fluid shear stress induces a distinct set of endothelial cell genes, most specifically lung Kruppel-like factor (KLF2). Blood 100 (5), 1689-1698.

201. Lu, L. et al. (2004) Activation of nuclear factor-kappaB and its proinflammatory mediator cascade in the infarcted rat heart. Biochem Biophys Res Commun 321 (4), 879-885

202. Hajra, L. et al. (2000) The NF-kappa B signal transduction pathway in aortic endothelial cells is primed for activation in regions predisposed to atherosclerotic lesion formation. Proc Natl Acad Sci U S A 97 (16), 9052-9057.

203. Rodriguez, C. et al. (2001) LDL downregulates CYP51 in porcine vascular endothelial cells and in the arterial wall through a sterol regulatory element binding protein-2-dependent mechanism. Circ Res 88 (3), 268-274.

References

Aird WC: 2003a. Endothelial cell heterogeneity. Crit Care Med 31:S221–S230.

Aird WC: 2003b. Targeting the endothelium in sepsis and multiorgan dysfunction. Sci Med 9:108–119.

Aird WC, Edelberg JM, Weiler-Guettler H, et al.: 1997. Vascular bed-specific expression of an endothelial cell gene is programmed by the tissue microenvironment. J Cell Biol 138:1117–1124.

Aird WC, Jahroudi N, Weiler-Guettler H, et al.: 1995. Human von Willebrand factor gene sequences target expression to a subpopulation of endothelial cells in transgenic mice. Proc Natl Acad Sci USA 92: 4567–4571.

Aitsebaomo J, Kingsley-Kallesen ML, Wu Y, et al.: 2001. Vezf1/DB1 is an endothelial cell-specific transcription factor that regulates expression of the endothelin-1 promoter. J Biol Chem 276:39197–39205.

Arap W, Kolonin MG, Trepel M, et al.: 2002. Steps toward mapping the human vasculature by phage display. Nat Med 8:121–127.

Chi JT, Chang HY, Haraldsen G, et al.: 2003. Endothelial cell diversity revealed by global expression profiling. Proc Natl Acad Sci USA 100:10623–10628.

Cines DB, Pollak ES, Buck CA, et al.: 1998. Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood 91:3527–3561.

Contrino J, Hair G, Kreutzer DL, Rickles FR: 1996. In situ detection of tissue factor in vascular endothelial cells: correlation with the malignant phenotype of human breast disease. Nat Med 2:209–215.

Crawley J, Lupu F, Westmuckett AD, et al.: 2000. Expression, localization, and activity of tissue factor pathway inhibitor in normal and atherosclerotic human vessels. Arterioscler Thromb Vasc Biol 20:1362–1373.

Drake TA, Cheng J, Chang A, Taylor FB.: 1993. Expression of tissue factor, thrombomodulin, and E-selectin in baboons with lethal *Escherichia coli* sepsis. Am J Pathol 142:1458–1470.

Edelberg JM, Tang L, Hattori K, et al.: 2002. Young adult bone marrow-derived endothelial precursor cells restore aging-impaired cardiac angiogenic function. Circ Res 90:E89–E93.

Evans V, Hatzopoulos A, Aird WC, et al.: 2000. Targeting the *Hprt* locus in mice reveals differential regulation of Tie2 gene expression in the endothelium. Physiol Genomics 2:67–75.

Guillot PV, Liu L, Kuivenhoven JA, et al.: 2000. Targeting of human eNOS promoter to the *Hprt* locus of mice leads to tissue-restricted transgene expression. Physiol Genomics 2:77–83.

Hatley ME, Srinivasan S, Reilly KB, et al.: 2003. Increased production of 12/15 lipoxygenase eicosanoids accelerates monocyte/endothelial interactions in diabetic db/db mice. J Biol Chem 278:25369–25375.

Kumar S, West DC, Ager A: 1987. Heterogeneity in endothelial cells from large vessels and microvessels. Differentiation 36:57–70.

Lacorre DA, Baekkevold ES, Garrido I, et al.: 2004. Plasticity of endothelial cells: rapid dedifferentiation of freshly isolated high endothelial venule endothelial cells outside the lymphoid tissue microenvironment. Blood 103:4164–4172.

Minami T, Donovan DJ, Tsai JC, et al.: 2002. Differential regulation of the von Willebrand factor and Flt-1 promoters in the endothelium of hypoxanthine phosphoribosyltransferase-targeted mice. Blood 100:4019–4025.

Minami T, Kuivenhoven JA, Evans V, et al.: 2003. Ets motifs are necessary for endothelial cell-specific expression of a 723-bp Tie-2 promoter/enhancer in Hprt targeted transgenic mice. Arterioscler Thromb Vasc Biol 23:2041–2047.

Nachman RL, Jaffe EA: 2004. Endothelial cell culture: Beginnings of modern vascular biology. J Clin Invest 114:1037–1040.

Nagayasu T, Saadi S, Holzknecht RA, et al.: 2000. Expression of tissue factor mRNA in cardiac xenografts: clues to the pathogenesis of acute vascular rejection. Transplantation 69:475–482.

Oh P, Li Y, Yu J, et al.: 2004. Subtractive proteomic mapping of the endothelial surface in lung and solid tumours for tissue-specific therapy. Nature 429:629–635.

Page C, Rose M, Yacoub M., Pigott R.: 1992. Antigenic heterogeneity of vascular endothelium. Am J Pathol 141:673–683.

Reese TS, Karnovsky MJ: 1967. Fine structural localization of a blood–brain barrier to exogenous peroxidase. J Cell Biol 34:207–217.

Schlaeger TM, Bartunkova S, Lawitts JA, et al.: 1997. Uniform vascular-endothelial-cell-specific gene expression in both embryonic and adult transgenic mice. Proc Natl Acad Sci USA 94:3058–3063.

Schlaeger TM, Qin Y, Fujiwara Y, et al.: 1995. Vascular endothelial cell lineage-specific promoter in transgenic mice. Development 121:1089–1098.

Solovey A, Kollander R, Shet A, et al.: 2004. Endothelial cell expression of tissue factor in sickle mice is augmented by hypoxia/reoxygenation and inhibited by lovastatin. Blood 104:840–846.

Turner RR, Beckstead JH, Warnke RA, Wood GS: 1987. Endothelial cell phenotypic diversity. In situ demonstration of immunologic and enzymatic heterogeneity that correlates with specific morphologic subtypes. Am J Clin Pathol 87:569–575.

Xiong JW, Leahy A, Lee HH, Stuhlmann H: 1999. Vezf1: a Zn finger transcription factor restricted to endothelial cells and their precursors. Dev Biol 206:123–141.

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