Early Growth Response-1 in Cardiovascular Pathobiology

Levon M. Khachigian

Abstract—The immediate-early gene product and zinc finger transcription factor early growth response (Egr-1) plays a key master regulatory role in multiple cardiovascular pathological processes. This article reviews the amassing recent evidence implicating Egr-1 in atherosclerosis, intimal thickening after acute vascular injury, ischemic pathology, angiogenesis, allograft rejection, and cardiac hypertrophy. (Circ Res. 2006;98:186-191.)

Key Words: Egr-1 ■ atherosclerosis ■ vascular injury ■ angiogenesis ■ ischemia and ischemia-reperfusion ■ allograft rejection ■ cardiac hypertrophy

E
arly growth response (Egr)-1,1 also known as NGFI-A,2 zif268,3 krox-24,4 and TIS85, is a transcription factor and product of an immediate-early gene located on human chromosome 5q23-q31 encoding 2 exons.1 The DNA binding domain of Egr-1 contains 3 zinc fingers of the Cys2-His2 subtype that bind preferentially to GC-rich elements.6 Egr-1 is poorly expressed in the normal artery wall but is activated by acute mechanical injury7 and other vascular stresses such as angiotensin II,8 lysophosphatidylcholine,9 PDGF,10 FGF-1,11 FGF-2,12 and fluid shear stress.13 Transcription of the Egr-1 gene is dependent on Ras-Raf-MEK-ERK1/2 pathway signaling (which is itself activated by acute vascular injury8,12,14,15) and serum-response elements in the Egr-1 promoter.16 These elements mediate Egr-1 inducibility in response to a variety of agonists, including thrombin,17 lipopolysaccharide,18 and hypoxia,19 and, as in the c-Fos promoter, involve interactions between ternary complex factors (TCF) and serum response factor (SRF).20 Recent studies suggest that nuclear accumulation of Egr-1 appears to require interaction of Gab1 (growth factor receptor-bound protein 2 [Grb2]-associated binder-1) with ERK1/2 via the proline-rich MET-binding domain in Gab1.21

Egr-1 is a master regulator because it controls the expression of a wide variety of genes. Oligonucleotide-based microarray analysis provided insights into the spectrum of these Egr-1-dependent genes. Expression of more than 300 genes was altered (229 upregulated, 74 downregulated) 3-fold or more 48 hours following human umbilical vein endothelial cell (HUVEC) infection with an adenovirus-driven form of Egr-1 resistant to transcriptional repression by its endogenous inhibitor NAB.22 These genes may broadly be grouped as transcription factors (eg, Egr-1, NAB1), signaling factors (eg, Notch3, Rad), growth factors and cytokines (eg, TGF-β, CLF1), cell-cycle regulators (eg, cyclin D1, p57kip2, matrix proteins [eg, fibronectin, osteopontin]) and ion channel regulators (eg, Na+/Ca2+-exchanger).22

Egr-1–dependent gene expression involves functional cooperativity (positive and negative) between Egr-1 and a growing number of other transcription or regulatory factors including nuclear factor of activated T cells (NFAT),23,24 steroidogenic factor-1 (SF-1),25,26 AP-2 and the glucocorticoid receptor,27 p300,25 RelA (p65),28,29 p53,30 Sp131 as well as NAB (NAB132 and NAB233). It is therefore quite likely that Egr-1 does not act alone, but works in concert with other transcription factors. We described functional interplay between Egr-1 and Sp1 in the context of PDGF-A34 and PDGF-B7 promoters, now recognized as a more general regulatory circuit in inducible gene expression. Examples of other genes that may be controlled by Egr-1/Sp1 exchange include the HGF receptor,36 RET protooncogene,37 tissue factor,38 and 5-lipoxigenase.39

This article reviews the growing evidence implicating Egr-1 in the control of a wide variety of cardiovascular pathological processes (Figure).

Atherosclerosis

Egr-1 contributes to the pathogenesis of atherosclerotic lesions. McCaffrey et al performed cDNA array and RT-PCR analysis comparing tissue from the dissected fibrous cap of human lesions with the underlying tunica media of patients with obstructive carotid atherosclerosis and revealed 5-fold higher levels of Egr-1 in lesions.40 Egr-1 was expressed mainly in α-SM-actin–positive cells in areas of macrophage infiltration, as well as in endothelial cells.40 Subsequent studies comparing Egr-1 protein expression in cells derived from the human atherosclerotic lesion and media at low passage revealed greater expression in lesions.41
Intimal Thickening After Acute Vascular Injury

Almost a decade ago we demonstrated by in situ hybridization that Egr-1 is rapidly (within hours) and transiently induced at the endothelial wound edge following balloon catheter scrape injury to the aortae of rats.7 In the same (endothelial denudation) model, we found that Egr-1 is expressed later by migrating SMCs.10 The expression and nuclear translocation of Egr-1 after mechanical injury is, at least in vitro, dependent on the release of endogenous FGF-2 and its paracrine activation of MEK/ERK-dependent signaling.12 FGF-2, a potent agonist of Egr-1, plays an important role vascular repair after injury. FGF-2 is locally and transiently (within minutes) released in the coronary arteries of humans undergoing stenting.48 SMC migration in rats after deendothelialization is augmented by systemic administration of FGF-2 and blocked by infusion of neutralizing antibodies to FGF-2.49

That Egr-1 plays a critical role as a mediator of SMC growth and intimal thickening in the reparative response to vascular injury has been demonstrated using a variety of synthetic nucleic acid-based gene-silencing approaches. For example, antisense oligonucleotides (15-mers) with phosphorothioate linkages targeting various regions in rat Egr-1 mRNA, unlike random, scrambled, sense or mismatch versions, blocked primary aortic SMC replication and regrowth after in vitro scraping injury.50 Catalytic 34-mer DNA molecules (DNAzyme ED5) which target and degrade rat Egr-1 mRNA, also inhibited SMC growth and repair in vitro and, when delivered adventitiously to rat common carotid arteries, suppressed Egr-1 protein expression after 1 hour and intimal thickening 14 days after balloon angioplasty.51 ED5 also blocks neointima formation in rat carotid arteries 18 days after permanent ligation.52 The DNAzyme has since been used to suppress the development of other proliferative states. For example, ED5 reduces cortical Egr-1 mRNA expression and interstitial fibrosis in rats 7 days after renal obstruction (unilateral ureteral ligation) and delivery by electroporation. In this model, the DNAzyme inhibited TGF-β expression of Egr-1–dependent genes, such as PDGF-B, and its paracrine activation of MEK/ERK-dependent signal- ing,49 transiently (within minutes) released in the coronary arteries of humans undergoing stenting.48 SMC migration in rats after deendothelialization is augmented by systemic administration of FGF-2 and blocked by infusion of neutralizing antibodies to FGF-2.49

That Egr-1 plays a critical role as a mediator of SMC growth and intimal thickening in the reparative response to vascular injury has been demonstrated using a variety of synthetic nucleic acid-based gene-silencing approaches. For example, antisense oligonucleotides (15-mers) with phosphorothioate linkages targeting various regions in rat Egr-1 mRNA, unlike random, scrambled, sense or mismatch versions, blocked primary aortic SMC replication and regrowth after in vitro scraping injury.50 Catalytic 34-mer DNA molecules (DNAzyme ED5) which target and degrade rat Egr-1 mRNA, also inhibited SMC growth and repair in vitro and, when delivered adventitiously to rat common carotid arteries, suppressed Egr-1 protein expression after 1 hour and intimal thickening 14 days after balloon angioplasty.51 ED5 also blocks neointima formation in rat carotid arteries 18 days after permanent ligation.52 The DNAzyme has since been used to suppress the development of other proliferative states. For example, ED5 reduces cortical Egr-1 mRNA expression and interstitial fibrosis in rats 7 days after renal obstruction (unilateral ureteral ligation) and delivery by electroporation. In this model, the DNAzyme inhibited TGF-β expression of Egr-1–dependent genes, such as PDGF-B, and its paracrine activation of MEK/ERK-dependent signaling,49 transiently (within minutes) released in the coronary arteries of humans undergoing stenting.48 SMC migration in rats after deendothelialization is augmented by systemic administration of FGF-2 and blocked by infusion of neutralizing antibodies to FGF-2.49

That Egr-1 plays a critical role as a mediator of SMC growth and intimal thickening in the reparative response to vascular injury has been demonstrated using a variety of synthetic nucleic acid-based gene-silencing approaches. For example, antisense oligonucleotides (15-mers) with phosphorothioate linkages targeting various regions in rat Egr-1 mRNA, unlike random, scrambled, sense or mismatch versions, blocked primary aortic SMC replication and regrowth after in vitro scraping injury.50 Catalytic 34-mer DNA molecules (DNAzyme ED5) which target and degrade rat Egr-1 mRNA, also inhibited SMC growth and repair in vitro and, when delivered adventitiously to rat common carotid arteries, suppressed Egr-1 protein expression after 1 hour and intimal thickening 14 days after balloon angioplasty.51 ED5 also blocks neointima formation in rat carotid arteries 18 days after permanent ligation.52 The DNAzyme has since been used to suppress the development of other proliferative states. For example, ED5 reduces cortical Egr-1 mRNA expression and interstitial fibrosis in rats 7 days after renal obstruction (unilateral ureteral ligation) and delivery by electroporation. In this model, the DNAzyme inhibited TGF-β expression of Egr-1–dependent genes, such as PDGF-B, and its paracrine activation of MEK/ERK-dependent signaling,49 transiently (within minutes) released in the coronary arteries of humans undergoing stenting.48 SMC migration in rats after deendothelialization is augmented by systemic administration of FGF-2 and blocked by infusion of neutralizing antibodies to FGF-2.49

Pathologic stimuli

Growth factors, cytokines, shear stress, injury

MAP kinases
Srf/TCF/SRE

Inducible transcriptional programs

• Atherosclerosis
• Restenosis
• Cardiac hypertrophy
• Ischemic disease
• Allograft rejection
• Angiogenesis

Others

Egr-1
HIF-1β
Ets-1
NFκB
JUN
ERK
ERK
PDGF-A, B, C
TGFβ, FGF-1
FGF-2, ICAM-1
VCAM-1,
IL-1β, MIP-1, uPA,
tissue factor, others

Schematic depicting various cardiovascular pathological processes thought to be under transcriptional control of the inducible immediate-early gene product and zinc finger nuclear regulatory factor, Egr-1. Egr-1 is activated by multiple pathophysiological stimuli involving mitogen-activated protein kinases and SRE-dependent transcription. Egr-1, once induced, as a master regulator, controls effector genes implicated in a myriad of cardiovascular conditions such as lesion development, hypertrophy, ischemia, and angiogenesis. Egr-1 is but 1 of many transcription factors involved in these processes and its interactions with these other factors are yet to be delineated.

Egr-1 is expressed in SMCs of atherosclerotic lesions in LDL receptor–deficient mice fed a high-fat diet for 5 weeks. Egr-1 staining intensity in lesions increased progressively by 10 and 20 weeks.40 observations supported by studies in apolipoprotein E (apoE)-null mice.42 Lesion area in mice deficient in both Egr-1 and apoE is dramatically reduced compared with mice lacking apoE alone. Female 24-week-old double-knockout mice fed a normal chow diet had 7-fold smaller lesions than apoE-null mice.42 Egr-1 is also found in inflammatory cells of vascular lesions. For example, Egr-1 is expressed in CD68+/H11001 macrophages of aortic atherosclerotic lesions in fructose-fed LDL receptor–deficient mice.43 In innominate arteries of apoE−/− mice, Egr-1 was primarily colocalized with macrophages in the lateral margins of advanced atherosclerotic lesions.44 Thus Egr-1 is found in several cell types within atherosclerotic lesions and is a factor required in atherogenesis. Egr-1 may also play a role in the pathophysiology of stenosed calcific valvular disease. Egr-1 has been detected in calcific human aortic valve cusps compared with noncalcified normal cusps.45

Egr-1 expression in lesions can be reduced with standard pharmacological agents. For example, simvastatin (50 mg/kg/d), a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, inhibits Egr-1 protein expression in lesions of apoE-null mice by more than 60%.44 This was accompanied by reduced tissue factor expression and fewer macrophages in the lesion.44 Chlamydia pneumoniae, a bacterium implicated in atherogenesis, can induce Egr-1 mRNA and protein expression in SMCs and macrophages within 1 hour apparently via ERK signaling.54,55 siRNA targeting human EGR-1 blocks C pneumoniae inducible DNA synthesis after 48 hours.46
targeting other transcription factors, such as NFAT,\textsuperscript{57} STAT3,\textsuperscript{58} nuclear factor \( \kappa B \),\textsuperscript{59} and c-Jun,\textsuperscript{60} that are also activated by arterial injury.\textsuperscript{57,58,60,61} suggests that the possibility of compound inhibition of postangioplasty restenosis in strategies targeting multiple factors.

**Hypoxia and Ischemia**

Understanding transcriptional changes in the brain after ischemia may provide therapeutic molecular targets for the treatment of stroke, a consequence of atherosclerosis. Several lines of evidence identify Egr-1 as a hypoxia-inducible factor. Gubits et al showed that unilateral ligation of the common carotid artery in 7-day-old pup rats followed by 3 hours of hypoxia (8% \( O_2 \)) results in increased Egr-1 mRNA expression in the ipsilateral forebrain within a few hours.\textsuperscript{62} DNA microarray studies later demonstrated that Egr-1 is upregulated in the hippocampal CA1 sector in rats 2 minutes after global cerebral ischemia.\textsuperscript{63} Finally, Egr-1 and Egr-1–dependent genes (such as PAI-1, ICAM-1, IL-1\( \beta \), and MIP-1) are induced in the perifraction cortex of adult rats 24 hours after permanent middle cerebral artery occlusion.\textsuperscript{64} Egr-1 is also upregulated in the kidney in response to renal artery occlusion.\textsuperscript{55,66}

Egr-1 appears to be involved in the pathogenesis of ischemic lung tissue damage. In a mouse model of lung ischemia and ischemia-reperfusion, Egr-1 (but not Sp1) was induced 3-fold within 15 minutes and approximately 10-fold after 1 hour, then induced 20-fold after 3 hours of reperfusion.\textsuperscript{67} That Egr-1–deficient mice display increased survival and lung function after pulmonary ischemia-reperfusion demonstrates a causal relationship between Egr-1 and ischemia-reperfusion damage.\textsuperscript{67} Egr-1–deficient animals had lower expression of genes such as tissue factor, PAI-1, and ICAM-1, suggesting Egr-1 governance of procoagulant and proinflammatory genes during ischemia-reperfusion injury. Recent studies show that Egr-1 expression, ERK, and JNK phosphorylation after lung ischemia-reperfusion is reduced in PKC-\( \beta \) knockout mice or wild-type mice fed the PKC-\( \beta \) inhibitor ruboxistaurin,\textsuperscript{68} demonstrating the importance of this PKC isofrom in Egr-1 signaling. Hypoxia induces Egr-1 expression in endothelial cells via the PKC-\( \alpha \)-mediated Ras/Raf-1/ERK1/2 pathway.\textsuperscript{69}

cDNA array analysis further showed that Egr-1 mRNA levels in rat lungs increase 5-fold 1 hour after warm ischemia following surgical extirpation compared with controls.\textsuperscript{70} Chronic exposure (14 days) of newborn calves to 12%, rather than 21%, \( O_2 \) upregulates Egr-1 protein expression in pulmonary artery adventitial fibroblasts. Hypoxia (1%) induces Egr-1 expression and DNA synthesis in fibroblasts cultured from hypoxic animals, after 6 and 24 hours, respectively. Hypoxia-inducible fibroblast proliferation, cyclin D, and epidermal growth factor receptor expression is inhibited after 48 hours.\textsuperscript{71} Interestingly, Egr-1 mRNA is increased 2-fold in the lung and 6-fold in the rat heart 6 hours following exposure to 0.1% carbon monoxide.\textsuperscript{72} Egr-1 is activated in response to cardiac ischemia. For example, Egr-1 mRNA is expressed after cardiopлегic arrest and reperfusion in human hearts and in rat hearts subjected to cold cardioplegia for 40 minutes followed by 40 minutes of reperfusion.\textsuperscript{73} Egr-1 expression is thus regulated by ischemic and ischemic-reperfuasive stress in multiple cell and tissue types.

**Cardiac Hypertrophy**

A role for Egr-1 in cardiac hypertrophy was suggested by the capacity of norepinephrine and phenylephrine to stimulate Egr-1 in rat hearts.\textsuperscript{74} Recently, Buitrago et al\textsuperscript{75} demonstrated that NAB1 and Egr-1 serve as endogenous regulators of pathologic cardiac hypertrophy. Left ventricular myocardial NAB1 expression increased in \( \beta_1 \)-adrenergic receptor transgenic mice, a well-established model of heart failure. Adenovirus-driven NAB1 suppressed cardiomyocyte hypertrophy, skeletal organization, and protein synthesis, whereas cardiomyocyte growth was unaffected by a mutant form of NAB1 that lacked the Egr-binding site. Although adrenergic stimulation led to ventricular hypertrophy in wild-type mice, this was significantly reduced in NAB1-overexpressing mice. Complementing these observations, ventricular and cardiac hypertrophy were reduced in Egr-1–null mice subjected to transverse aortic constriction-induced pressure overload. In contrast, physiologic hypertrophy induced by exercise was unaffected in NAB1 transgenic mice compared with wild-type animals, suggesting a role in pathologic but not physiological hypertrophy.\textsuperscript{75} Saadane et al by contrast showed no change in total ventricular weight in Egr-1–deficient mice after chronic infusion of isoproterenol/phenylephrine compared with wild type mice, despite dramatic differences in adrenoreceptor-stimulated expression of genes such as GATA-4 and Nkx2.5.\textsuperscript{76}

**Allograft Rejection**

Egr-1 also appears to play a role in cardiac allograft rejection. Egr-1 mRNA and protein were detected in leukocytes and cardiac myocytes of grade 3 endomyocardial biopsies but not in grade 0 biopsies. Egr-1 is expressed in the coronaries of patients with coronary artery vasculopathy (CAV).\textsuperscript{77} These findings are supported by observations of Egr-1 expression in SMCs of rejected cardiac allografts in monkeys undergoing heterotopic abdominal heart transplants.\textsuperscript{78} Egr-1 and Egr-1–dependent genes, such as ICAM-1, VCAM-1, and PDGF-A, are induced following transplantation of heterotopic murine cardiac allografts. In contrast, these genes are poorly expressed in homozygous Egr-1–deficient donor allografts, in which parenchymal rejection and CAV are suppressed.\textsuperscript{79} Antisense Egr-1 oligonucleotides delivered at the time of organ harvest inhibit Egr-1 expression and the development of CAV.\textsuperscript{79} Antisense Egr-1 also block lung transplant leukostasis, inflammation, and thrombosis in rats together with improved graft function and recipient survival.\textsuperscript{80} These findings thus implicate Egr-1 in vasculopathy and organ rejection.

**Angiogenesis**

Inducible expression of Egr-1 in activated vascular endothelium\textsuperscript{2} led us to investigate whether Egr-1 may play direct a role in the angiogenic process. Observations of poor neovascularization in subcutaneous Matrigel implants of Egr-1–deficient mice were consistent with suppressed implant angiogenesis in normal mice using Egr-1 DNAzymes. These agents inhibited microvascular endothelial cell proliferation.
Egr-1, like HIF86 is an inducible proangiogenic gene regulating FGF-2,81 Flt-1,84 tissue factor,83 and uPA83 in the context of angiogenesis. For example, Egr-1 controls the expression of receptors. For example, Egr-1 controls the expression of involucrin and other regulators, although this knowledge is still relatively limited. The influence of oxidation, phosphorylation, acetylation, and deacetylation on Egr-1 transactivation and the specific disease contexts involved are also not clear. Future studies should address these deficiencies and extend the key pathophysiological roles of Egr-1 beyond rodent models, from where much of our current understanding on Egr-1 is largely derived, to larger animal systems. This will help direct which of the panoply of cardiovascular conditions possibly regulated by Egr-1 in humans could be targeted with Egr-1-specific strategies as primary or adjunctive therapies.

Concluding Remarks

Egr-1 is a master regulator that plays a key role in a variety of cardiovascular pathological processes such as atherosclerosis, intimal thickening following acute vascular injury, ischemia-reperfusion, cardiac hypertrophy, allograft rejection, and angiogenesis. Much of this knowledge has been derived from investigations using Egr-1–null mice or specific molecular suppressors of Egr-1, such as antisense oligonucleotides, decoys, siRNA, DNAzymes, or NABs. Egr-1 dynamically links changes in the local cellular environment with the altered expression of key genes mediating a broad spectrum of vascular pathologies. Egr-1 transcriptional activity involves functionally important interactions with NABs and other regulators, although this knowledge is still relatively limited. The influence of oxidation, phosphorylation, acetylation, and deacetylation on Egr-1 transactivation and the specific disease contexts involved are also not clear. Future studies should address these deficiencies and extend the key pathophysiological roles of Egr-1 beyond rodent models, from where much of our current understanding on Egr-1 is largely derived, to larger animal systems. This will help direct which of the panoply of cardiovascular conditions possibly regulated by Egr-1 in humans could be targeted with Egr-1–specific strategies as primary or adjunctive therapies.

Acknowledgments

The author thanks Dr Ravinay Bhindi and Gabriella Khachigian for helpful comments. Work in the laboratory of the author was supported by grants from the National Health and Medical Research Council, National Heart Foundation, Australian Research Council, and the New South Wales State Department of Health. The author is a Senior Principal Research Fellow of the National Health and Medical Research Council.

References


Roles of Egr-1 in Cardiovascular Pathobiology

Khachigian


