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Early growth response transcription factors: key mediators of fibrosis and novel targets for anti-fibrotic therapy

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Abstract

Fibrosis is a deregulated and ultimately defective form of tissue repair that underlies a large number of chronic human diseases, as well as obesity and aging. The pathogenesis of fibrosis involves multiple cell types and extracellular signals, of which transforming growth factor- β (TGF- β) is pre-eminent. The prevalence of fibrosis is rising worldwide, and to date no agents has shown clinical efficacy in the attenuating or reversing the process. Recent studies implicate the immediate-early response transcription factor Egr-1 in the pathogenesis of fibrosis. Egr-1 couples acute changes in the cellular environment to sustained alterations in gene expression, and mediates a broad spectrum of biological responses to injury and stress. In contrast to other ligand-activated transcription factors such as NF- κ B, c-jun and Smad2/3 that undergo post-translational modification such as phosphorylation and nuclear translocation, Egr-1 activity is regulated via its biosynthesis. Aberrant Egr-1 expression or activity is implicated in cancer, inflammation, atherosclerosis, and ischemic injury and recent studies now indicate an important role for Egr-1 in TGF- β -dependent profibrotic responses. Fibrosis in various animal models and human diseases such as scleroderma (SSc) and idiopathic pulmonary fibrosis (IPF) is accompanied by aberrant Egr-1 expression. Moreover Egr-1 appears to be required for physiologic and pathological connective tissue remodeling, and Egr-1-null mice are protected from fibrosis. As a novel profibrotic mediator, Egr-1 thus appears to be a promising potential target for the development of anti-fibrotic therapies.

Keywords

Egr-1; TGF- β ; fibrosis; Scleroderma (systemic sclerosis); fibroblast

Introduction

Fibrosis is characterized by relentless accumulation of collagen-rich extracellular matrix (ECM) that eventually results in disrupted tissue architecture and organ failure. Because fibrosis accounts for as much as 40% of all deaths worldwide and appears to be increasing in

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prevalence (Wynn, 2010), understanding its pathogenesis and developing therapeutic strategies to block its progression are urgent scientific challenges. Both the initiation and persistence of pathological fibrosis involve activation and differentiation of mesenchymal cells (Kalluri, 2009). Transforming growth factor- β (TGF- β) is a pivotal signal for triggering fibrogenic responses, generally acting in concert with hypoxia, reactive oxygen species (ROS), Wnt and Notch signaling, along with chemokines, cytokines and mechanical stress signals (Wei et al, 2010). TGF- β responses are mediated through both canonical (Smad-dependent) and Smad-independent intracellular signal transduction mechanisms (Rahimi et al, 2007). Early growth response-1 (Egr-1) is a novel Smad-independent mediator of TGF- β signaling. This zinc finger DNA-binding protein belongs to a family of ligand-inducible early response genes (Egrs) that are implicated in diverse physiological processes, and in the pathogenesis of multiple inherited and acquired human diseases. Emerging studies reveal a novel function for Egr-1 as an important mediator of TGF- β -induced responses. Moreover, abnormal Egr-1 expression and function has been linked to animal models of fibrosis, as well as human fibrotic disorders including idiopathic pulmonary fibrosis and scleroderma. This review focuses on recent insights into the regulation and complex functional role of Egr-1 and related proteins in fibroblast biology, connective tissue homeostasis, physiologic tissue repair and pathological fibrosis.

Egr-1 and the early growth response gene family

The Egr-1 gene, located on human chromosome 5q31, encodes an 80 kDa DNA-binding transcription factor (Khachigian and Collins, 1998). In resting cells, Egr-1 expression is low or undetectable. However, Egr-1 expression is elicited by a large number of extracellular stimuli, typically in a rapid and transient manner. The Egr-1 protein (shown in Fig. 1) consists of transactivation and repression domains, along with three DNA-binding zinc fingers that recognize GC-rich sequences present in target gene promoters (Gashler A and Sukhatme, 1995). An inhibitory domain, located between the activation domain and the DNA-binding domain serves as a recognition site for Egr-1/NGFI-A binding proteins 1 (Nab1) and Nab2 (Svaren et al., 1996; Russo et al, 1995). These cofactors repress Egr-1-dependent transcriptional activity via recruitment of the inhibitory nucleosomal remodeling and deacetylation (NuRD) complex to the promoter-bound Egr-1 (Srinivasan et al, 2006). The levels of Nab1/2 and the ratio of Egr-1/Nab1/2 in a particular cell are thus of extreme importance for setting the level of Egr-1 activity. The expression of Nab2 is itself controlled by Egr-1, enabling Egr-1 to regulate its own biological activity via a negative feedback loop (Ehrensgruber et al, 2000). Other members of the Egr family are listed in Table 1.

Egr-1 activity is controlled at the transcriptional level

Egr-1 activity is determined largely by regulation of its biosynthesis, which is under tight control. Egr-1 expression is induced by environmental stimuli characteristically associated with injury and stress. The list includes growth factors, cytokines, T cell receptor ligation, hormones, thrombin, shear stress and mechanical forces, neurotransmitters, ultraviolet light, reactive oxygen species (ROS), ischemia-reperfusion and hypoxia (Hjoberg et al, 2004; Yan SF, 2000; Gaggioli et al, 2005; Guha et al, 2001; Li et al, 2007; Kaufmann et al, 2002; Rossler et al, 2009; Lohoff et al, 2010).

Much is now known about how these extracellular stimuli induce the expression of Egr-1. The human Egr-1 gene promoter harbors five consensus serum response elements (SREs) that are recognized by serum response factor (SRF) and ternary complex factors (TCF) such as Elk-1 and related Ets family oncogenes (Buchwalter et al, 2004). The induction of Egr-1 characteristically involves the ERK kinase cascade converging on Elk-1 (Hasan et al, 2008; Kachigian, 2006; Midgley et al, 2004; Hjoberg et al, 2004). Phosphorylation enhances Elk-1

DNA binding activity, resulting in Egr-1 transcription. The Egr-1 gene promoter also contains an inhibitory Egr-1 binding site, which enables Egr-1 to negatively regulate its own expression (Cao et al, 1993). This feedback mechanism normally allows prompt extinction of Egr-1-dependent signaling.

Profibrotic signals induce Egr-1

We recently discovered that another potent stimulus for Egr-1 expression is TGF- β , which causes rapid and transient up-regulation of Egr-1 transcription in normal human fibroblasts via a Smad-independent MEK1-ERK1/2- Elk-1 pathway (Bhattacharyya et al, 2008). Moreover TGF- β enhanced the interaction of endogenous Egr-1 with the consensus Egr-1-binding element of the human COL1A2 promoter in vitro and in vivo (Chen et al, 2006). Insulin-like growth factor binding protein-5 (IGFBP-5), a member of IGFBP family that is upregulated in lung fibrosis, also induces Egr-1 via the MAPK signaling pathway (Beattie et al, 2006). Like TGF- β , IGFBP5 stimulates collagen production via Egr-1 (Yasuoka et al, 2009).

The range of Egr-1's biological activities induces fibrogenesis

Studies during the past two decades have implicated aberrant Egr-1 expression or function in carcinogenesis, inflammation, atherosclerosis, ischemic injury as well as fibrosis (Krones-Herzig et al, 2005; Tureyen et al, 2008; Du et al, 2000; Khachigian, 2006; Yan SF, 2000; Bhattacharyya et al, 2008; Wu et al, 2009). It was therefore a surprising observation that mice lacking Egr-1 appeared to show no overt phenotype, with the exception of infertility in females (Lee SL, 1996). Upon its induction by environmental signals, Egr-1 regulates genes involved in broad range of responses, including many that are important for fibrogenesis (Table 3). These include the profibrotic cytokine TGF- β , platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF) and vascular endothelial growth factor (VEGF), along with plasminogen activator inhibitor-1 (PAI-1), hemin, fibronectin, tissue inhibitor of metalloproteinase (TIMP1) and Osteopontin (Baron et al, 2006; Midgley et al, 2004; Theil and Cibelli, 2002; Hjoberg et al, 2004; Hasan et al, 2008; Guha et al, 2001; Yan et al, 2000; Freidrich et al, 2008; Yu et al, 2010). Moreover, in HepG2 cells, Egr-1 induces epithelial-mesenchymal transition (EMT), an important cellular response involved in fibrosis, by upregulating Snail (Grotegut et al, 2006). In normal skin and lung fibroblasts, forced expression of Egr-1 was sufficient to cause up-regulation of COL1A2 promoter activity, and further enhanced the stimulation induced by TGF- β (Chen et al, 2006). Fibroblasts lacking Egr-1 showed attenuated TGF- β responses despite intact Smad signaling, and forced expression of ectopic Egr-1 in these cells could restore TGF- β sensitivity (Chen et al, 2006). Subcutaneous administration of bleomycin in mice results in scleroderma-like dermal fibrosis and enhanced local TGF- β signaling (Wu and Varga, 2008). Bleomycin induced scleroderma was accompanied by increased Egr-1 accumulation in lesional fibroblasts (Bhattacharyya et al, 2008). The up-regulation of TGF- β signaling upon bleomycin treatment was attenuated in Egr-1-null mice. Treatment of wildtype mice with rosiglitazone, a PPAR- γ agonist, prevented dermal fibrosis (Wu et al, 2008). Remarkably, Egr-1 up-regulation in lesional skin was also prevented, implicating Egr-1 as a potential target for the anti-fibrotic effects of rosiglitazone.

In order to get an unbiased view of genes regulated by Egr-1 in fibroblasts, we performed microarray analysis. Genome-wide expression profiling identified 647 genes whose expression was significantly altered by Egr-1 (Bhattacharyya S, Du P et al, unpublished). Gene Ontology (GO) analysis (Fig. 3) showed that genes associated with cell proliferation, TGF- β signaling, wound healing, extracellular matrix synthesis (ECM), and vascular development were strongly represented in the "Egr-1 response gene signature". One of these

genes is the NADPH oxidase 4 (NOX4), which showed > eight-fold increase in Egr-1-expressing fibroblasts. NOX4 catalyzes the generation of superoxide and other ROS. NOX4 expression is strongly induced by TGF- β in a variety of cell types, and the consequent generation of ROS is implicated in mediating TGF- β responses including myofibroblast transformation (Hecker et al, 2009). Chronic hypoxia was shown to induce elevated NOX4 in pulmonary artery smooth muscle cells via Egr-1 (Djordjevic et al., 2005; Sturrock et al., 2006; Diebold I, 2010; Gupte and Wolin, 2008). In SSc patients, TGF- β and hypoxia together might be responsible for sustained Egr-1 expression, resulting in increased NOX4 activity and oxidative stress, which further exacerbates fibrogenesis (Gabrielli et al, 2009).

Deciphering profibrotic Egr-1 signaling: the roles of c-Abl and p300

Recent studies have yielded new insights into how Egr-1 mediates fibrotic responses. The c-Abl oncoprotein is a non-receptor tyrosine kinase that is constitutively activated in patients with chronic myelogenous leukemia (CML) (Rowley, 1973; Lugo et al, 1990). Mutated Bcr-Abl drives uncontrolled myeloid cell proliferation due to constitutive MEK1 phosphorylation (Kharbanda et al, 2000). It was recently shown that TGF- β directly induced the activity of c-Abl (Bhattacharyya, 2008; Wilkes and Leof, 2006). The response was specific for mesenchymal cells, and was not seen in epithelial cells. Ectopic c-Abl stimulated collagen gene expression, whereas its inhibition abrogated the stimulation by TGF- β . Moreover, pharmacological blockade of c-Abl in vivo using imatinib mesylate prevented fibrosis in animal models (Daniels et al, 2004; Wang et al, 2005; Wilkes and Leof, 2006; Distler et al, 2007). We found that in normal skin fibroblasts ectopic c-Abl directly induced Egr-1 expression, and c-Abl was both necessary and sufficient for Egr-1 stimulation (Bhattacharyya et al, 2009). Moreover, genetic and pharmacological approaches demonstrated that Egr-1 was also indispensable for c-Abl-induced collagen stimulation (Bhattacharyya et al, 2008). Together, these findings indicate that fibrotic TGF- β responses involve c-Abl stimulation of Egr-1, and the pathway is therefore amenable to disruption by small molecule kinase inhibitors such as imatinib. Indeed, this mechanistic model for TGF- β -induced fibrogenesis involving c-Abl accounts for the potent antifibrotic efficacy of imatinib in animal models, and provides the rationale for current clinical trials of c-Abl inhibitors in SSc and other fibrotic diseases (Daniels et al., 2004; Wang et al, 2005; Wilkes and Leof, 2006; Distler et al, 2007).

The phosphoprotein p300/CBP is a ubiquitously expressed transcriptional coactivator with diverse biological functions, including a critical role in Smad-dependent TGF- β responses (Ghosh and Varga, 2007). Overexpression of p300 is sufficient by itself to stimulate collagen gene expression and p300 further enhances Smad-dependent TGF- β responses, whereas loss of p300 results in attenuation (Ghosh et al, 2000; Bhattacharyya et al. 2005). We found that TGF- β stimulate p300 expression via Egr-1 (Ghosh et al, unpublished). This response is similar to serum induction of p300, which was also shown to be mediated via Egr-1 (Yu et al, 2004). These studies place c-Abl upstream of Egr-1 in the TGF- β fibrotic response, and show Egr-1 itself to be upstream of p300, the indispensable coactivator for Smad-dependent fibrotic gene expression (Fig. 2A).

Complex and dynamic cross-regulation of Egr family members in profibrotic TGF- β signaling

In addition to Egr-1, the early response gene family also includes Egr-2, Egr-3, and Egr-4, along with their inhibitors Nab1 and Nab2 which have complex and dynamic interactions with each other (Table 1). Members of the family play important roles in cell growth, differentiation, and apoptosis induced by environmental stimuli. While highly homologous,

the distinct Egr's show only partially overlaps patterns of tissue expression and biological activity.

Egr-2 is also induced by TGF- β and mediates profibrotic responses

Egr-2 plays important roles in immune regulation as an inhibitor of T cell activation, and is critical for maintaining immune tolerance (Collins et al, 2008). Mice with spontaneous lupus-like disease show diminished Egr-2 expression (Sela et al, 2008), and genetic targeting of Egr-2 in T cells results in expansion of autoreactive T cells, and loss of self tolerance (Zhu et al, 2008) #250}. Egr-2 is also essential for hindbrain development and peripheral nerve myelination, and in striking contrast to Egr-1-null mice, Egr-2-null mice suffer early lethality (Collins et al, 2008; Le et al, 2005). We recently began to investigate the distinct role of Egr-2 in fibrosis. We found that Egr-2 functions as an intracellular mediator of collagen gene stimulation and related fibrotic responses elicited by TGF- β (Feng et al, 2011). However, in contrast to Egr-1, which showed rapid up-regulation in response to TGF- β , stimulation of Egr-2 was delayed but sustained. Interestingly, Egr-1 itself induced Egr-2 expression. Ectopic expression of Egr-2 in normal fibroblasts directly induced the expression of a large number of profibrotic genes. Furthermore, Egr-2 level were found to be elevated in fibrotic skin and lung biopsies from patients with SSc. Thus, Egr-2 plays a distinct and functionally non-redundant role in ECM regulation and fibrogenesis.

The corepressor Nab2 blocks Egr-1-dependent TGF- β responses

A member of the Egr gene family Nab2 is a 55 kD nuclear protein, that was originally identified based on its ability to interact with Egr-1 in a yeast two-hybrid assay (Svaren et al, 1996). As a corepressor, Nab2 lacks DNA-binding activity, but can positively or negatively modulate the transcription of Egr-1 target genes via direct interaction with Egr-1 (Svaren et al, 1998). Many of the same environmental signals inducing Egr-1, also stimulates Nab2 expression (Bhattacharyya et al, 2009). Overexpression of Nab2 expression in normal fibroblasts blocked Egr-1-dependent transcription, and prevented the stimulation of collagen synthesis and myofibroblasts differentiation induced by TGF- β (Bhattacharyya et al, 2009). Inhibition involved HDAC1 recruitment to the COL1A2 promoter, and was accompanied by reduced histone H4 acetylation at the COL1A2 locus (Fig. 2B). A physiologic role for Nab2 in suppressing TGF- β signaling is indicated by loss-of- function studies: mice with targeted deletion of Nab2 displayed increased collagen deposition in the dermis, and Nab2^{-/-} MEFs showed constitutively elevated collagen synthesis (Bhattacharyya et al, 2009). Therefore, Nab2 appears to be a novel endogenous inhibitor of TGF- β activity via negative regulation of Egr-1-dependent signaling. A recent study highlighted the complex relationship between the Egr's and Nab2 by demonstrating that Egr-1, Egr-2, and Egr-3 can each activate Nab2 transcription which is in turn repressed by Nab2, thus establishing a cellular context-dependent negative feedback loop (Kumbrink et al, 2010)

Egr-1 implicated in fibrosis: insights from animal models

The key role of Egr-1 in fibrosis is highlighted by recent studies showing aberrant expression in several animal model of fibrosis. For instance, elevated Egr-1 was noted in the lungs of transgenic mice expressing TGF- β (Lee et al, 2004) or IL-13 (Cho et al, 2006), and in the gut in a mouse model of Crohn's disease (Fichtner-Feigl et al, 2008). We showed that the expression of Egr-1 was elevated in lesional skin from mice with bleomycin-induced scleroderma, with expression localized principally to fibroblastic cells in the dermis (Bhattacharyya et al, 2008). On the other hand, Egr-1 deficiency blunted the development of cardiac hypertrophy induced by catecholamine (Saadane et al, 2000). We recently showed that Egr-1 null mice were protected from bleomycin-induced skin and lung fibrosis (Fig. 4) (Wu et al, 2009). Curiously, recent studies indicated that TGF- α -induced lung fibrosis and

CCl4-induced hepatic fibrosis appeared to be exacerbated, rather than attenuated, in mice lacking Egr-1 raising the possibility that Egr-1 in fact has a more context-dependent role in fibrogenesis, possibly determined by the initiating nature of injury and cell type where it is active (Kramer et al, 2009; Pritchard et al, 2010). To further investigate the role of Egr-1 in fibrosis, we generated transgenic mice with fibroblast-specific Egr-1 overexpression by using a 6-kb enhancer from the pro 2(I) chain of the mouse type I collagen (Col1a2) gene. These transgenic mice did not develop spontaneous fibrosis, but did show a robust and exaggerated healing of incisional wounds (Wu et al, 2009).

Aberrant Egr-1 regulation and expression in human fibrosing disorders

Emerging evidence implicates aberrant Egr-1 expression in human fibrotic disorder. Elevated Egr-1 expression has been demonstrated in atherosclerotic plaques where Egr-1 levels correlated with collagen accumulation (Bot et al, 2009). Elevated Egr-1 expression has also been reported in lesional skin and lung biopsies from patients with diffuse SSc (Fig. 5) and lungs of patients with idiopathic pulmonary fibrosis (IPF) (Bhattacharyya et al, 2008; Yasuka et al, 2009). Genome-wide expression profiling comparing lungs from IPF patients with stable versus rapidly progressive disease showed elevated Egr-1 expression to be associated with rapid progression (Boon et al, 2009). Intriguingly, elevated Egr-1 gene expression in peripheral blood cells from SSc patients was associated with pulmonary arterial hypertension in patients with SSc (Grigoryev et al, 2008).

Further evidence implicating Egr-1 signaling in SSc comes from recent DNA microarray studies. The “Egr-1 responsive gene signature” defined by 647 genes whose expression was significantly regulated by Egr-1 in normal fibroblasts was strongly associated with skin biopsies from the ‘diffuse-proliferation’ subset of SSc patients (Milano et al, 2008; Sargent et al, 2009; Bhattacharyya S, Sargent J et al, Ms. in preparation).

Summary and perspectives

Emerging insights from in vitro experiments, transgenic animal models and human diseases point to an important novel role for Egr-1 in physiological and pathological tissue repair and fibrosis. Egr-1 expression is induced by fibrogenic stimuli, and Egr-1 in turn can regulate the expression of extracellular matrix components, matrix remodeling enzymes and fibrogenic cytokines such as TGF- β , and drive myofibroblast differentiation and EMT. Egr-1 shows persistent elevation in fibrotic lesions in a variety of human fibrosing diseases, and in animal models of fibrosis. Microarray studies reveal the “Egr-1-responsive gene signature” in skin biopsies from SSc patients in the ‘diffuse-proliferation’ subset (Bhattacharyya et al, unpublished). Moreover, elevated Egr-1 expression in the lungs predicts rapid progression of fibrosis in IPF patients (Boon et al, 2009).

The identification of novel signaling pathways and mediators that are altered in fibrosis and contribute to tissue damage opens the door to the development of strategies for their selective targeting. Blocking Egr-1 expression or biological activity therefore appears to be a potential novel approach to control pathological fibrogenesis. It is noteworthy that several drugs in current clinical use display potent inhibitory activity for Egr-1 induction or activity. These include mycophenolate mofetil, cyclosporine, simvastatin, imatinib mesylate, and insulin-sensitizing peroxisome proliferator activated receptor- γ ligands such as rosiglitazone (Farivar et al, 2005; Bea et al 2003; Bhattacharyya et al 2008; Okada et al, 2002; Wu et al 2009). Some of these agents have already shown efficacy in animal models of fibrosis, and may be candidates in the treatment of SSc, IPF and other fibrotic conditions.

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Fig. 1.

Egr-1 and related proteins of the Egr gene family. Egr-1 consists of activation domains, an inhibitory (Nab Interaction) domain and a DNA-binding domain.

Fig.2A

Fig. 2B

Fig. 2.

Regulation of TGF- β responses by Egr-1 and its inhibitor. A. TGF- β binds to the T β R1–T β R2 complex causing activation of Smad2/3. The pSmad2/3-Smad4 complex then translocates into the nucleus, where it binds to Smad binding elements (SBE) of target genes. The coactivator p300 is recruited to the Smad complex to activate COL1A2 transcription. TGF- β also activates a non-Smad pathway via c-Abl which in turn stimulates Egr-1 via MAP kinase. Egr-1 then binds to the Egr-1 binding element (EBS) to further stimulate COL1A2 transcription. B: Nab2 modulates Egr-1 activity and TGF- β signaling. In unstimulated fibroblasts (basal state), small amounts of Egr-1 and Nab2 are constitutively associated with the COL1A2 promoter (left upper panel). During early TGF- β stimulation (30min–4h) (right upper panel), Egr-1 is induced and Egr-1 is recruited to the COL1A2 promoter, where it enhances histone H4 hyperacetylation and stimulates transcription. Sustained TGF- β stimulation (2–48h) (left lower panel) leads to increased Nab2 expression and its accumulation in the Egr-1-COL1A2 transcriptional complex, where recruitment of HDAC1 resulting in H4 histone deacetylation and transcriptional silencing. In pathological fibrosis associated with constitutive Egr-1 expression (exemplified by scleroderma fibroblasts), defective Nab2 induction or function might results in unopposed Egr-1 signaling and target gene transcription (right lower panel).

Fig. 3. Normal human dermal fibroblasts were infected with Ad-EGFP or Ad-Egr-1m (100 MOI). At the end of 24 or 48 h incubation, total RNA was isolated and subjected to genomewide transcriptional analysis using Illumina Microarray chips. Genome-wide expression profiling of Egr-1 regulated genes. GO analysis showing biological processes significantly enriched with Egr-1 regulated genes ($p < 0.001$). A subset of the biological processes is shown.

Fig. 4. Egr-1 modulates lung fibrosis in mice and human. A. Reduced lung fibrosis in mice lacking Egr-1. Egr-null mice and wild-type littermates received daily s.c. injections of bleomycin or PBS for 14 days. Fourteen days after the last injections, the lungs were harvested and examined with Masson's trichrome stain (Original magnification x400). B. Elevated Egr-1 expression in SSc lung. Lesional lung tissue from patients with SSc- associated end-stage fibrosis (n=3) and healthy controls (n=3) were examined by immunohistochemistry with antibodies to Egr-1. Representative images are shown (original magnification x1000).

Table 1

Biological activities regulated via Egrs.

Gene	Prominent Functions	Mouse Knockout phenotype	Disease associations
Egr-1	Growth, development, differentiation, apoptosis, inflammation, fibrosis	Homozygous Egr-1 null overtly healthy; reduced body size; females infertile	Prostate cancer, lung cancer, breast cancer, schizophrenia, SSc
Egr-2	Formation and maintenance of peripheral nerve myelin, hindbrain development, T cell regulation, apoptosis	Homozygous Egr-2 null had congenital hypomyelinating neuropathy; uniformly die within days after birth	Charcot-Marie-Tooth disease, Dejerine-Sottas syndrome, congenital hypomyelinating neuropathy, SLE, schizophrenia, SSc
Egr-3	Sympathetic nervous system development, regulation of T-cell activation, survival and proliferation	Egr-3 null mice had profound ataxia; lack of muscle spindle	607A-G SNP associated with Schizophrenia
Egr-4	Central nervous system function (not well known)	Homozygous Egr-4 null overtly healthy; males infertile	Unknown
Nab1 [*]	Peripheral nerve differentiation, cardiac hypertrophy	Nab1-null mice produced at the expected Mendelian ratios, overtly healthy.	Unknown
Nab2 [*]	Vascular homeostasis, differentiation of endothelial cells, fibroblasts, inflammation, peripheral nerve myelination	Nab2 null overtly healthy; thick dermis; increased collagen accumulation	Unknown

* Nab1/Nab2 double null mice have severe hypomyelination and suffer early lethality SSc, Scleroderma; SLE Systemic Lupus Erythematosus; SNP, single nucleotide polymorphisms