

PATHOBIOLOGY IN FOCUS

Role of serum response factor in the pathogenesis of disease

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Serum response factor (SRF) is a ubiquitously expressed transcription factor that binds to a DNA *cis* element known as the CARG box, which is found in the proximal regulatory regions of over 200 experimentally validated target genes. Genetic deletion of SRF is incompatible with life in a variety of animals from different phyla. In mice, loss of SRF throughout the early embryo results in gastrulation defects precluding analyses in individual organ systems. Genetic inactivation studies using conditional or inducible promoters directing the expression of the bacteriophage Cre recombinase have shown a vital role for SRF in such cellular processes as contractility, cell migration, synaptic activity, inflammation, and cell survival. A growing number of experimental and human diseases are associated with changes in SRF expression, suggesting that SRF has a role in the pathogenesis of disease. This review summarizes data from experimental model systems and human pathology where SRF expression is either deliberately or naturally altered.

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Genomic DNA is a quaternary code comprising protein-coding and non-protein-coding sequences. While the protein-coding sequences are well-defined and increasingly understood, we are only beginning to elucidate the hidden information within the vast landscape of the non-protein-coding sequences. The field of comparative genomics has facilitated the identification of transcription factor-binding sites (TFBS) and microRNAs (miRs), which, aside from repetitive DNA sequences, are among the more easily decipherable non-protein-coding sequences in the human genome. Together, TFBS and miRs have essential roles in cell fate determination and cellular homeostasis of most life forms. Sequence variations (eg, single-nucleotide polymorphisms, SNPs) in the TFBS, miRs, and miR target sequences alter gene/protein expression and thus disturb homeostasis leading to disease.^{1–4} A major imperative, therefore, is decoding the non-protein-coding genome relating to gene regulation to gain a full understanding of how DNA-binding transcription factors and the estimated one million or more TFBS direct normal biological processes.

Serum response factor (SRF) is the founding member of the MADS-box family of transcription factors⁵ and is one of the best understood DNA-binding proteins in the human

proteome. The DNA-binding properties of SRF and its molecular cloning were first defined in the laboratory of Richard Treisman.^{6,7} SRF has relatively low intrinsic transcriptional activity, but its interaction with over 60 cofactors confers strong transactivation potential in a cell- and context-specific manner. At least two major signaling pathways converge upon SRF to direct the programs of gene expression.^{8,9} The classic pathway involves growth factor stimulation and mitogen-activated protein kinase signaling leading to the phosphorylation of the SRF cofactor, ELK1, and the activation of growth-related genes.^{10,11} The second regulatory pathway occurs through Rho-dependent changes in actin dynamics.¹² In this pathway, signal inputs lower the ratio of globular actin to fibrillar actin thereby liberating the binding of myocardin-related transcription factor-A (MRTF-A)/MAL to globular actin resulting in nuclear accumulation of MAL and subsequent SRF-dependent gene expression.¹³ Myocardin is a powerful SRF cofactor that directs the expression of smooth muscle- and cardiac muscle-specific contractile genes.^{14,15} Myocardin is related to MRTF-A, but does not undergo nucleocytoplasmic shuttling because its N-terminal actin-binding domains have a much lower affinity for globular actin than those found in MRTF-A.¹⁶ Both

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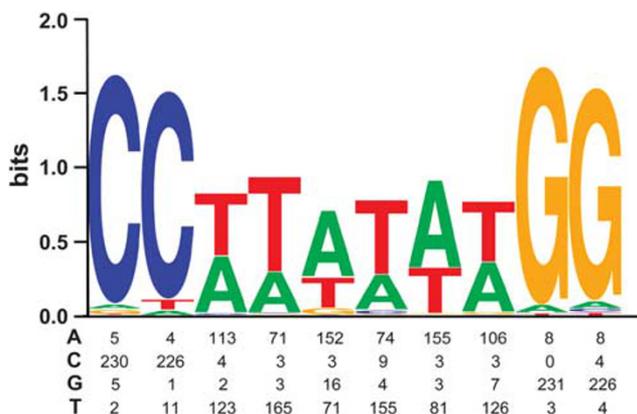


Figure 1 The CArG box. Shown is a sequence logo representing 242 conserved CArG boxes from 182 experimentally validated SRF-target genes (some genes contain > 1 CArG box). The height of each stack of nucleotides reflects conservation at the specified position measured in 'bits'.¹⁰⁶ The height of each nucleotide within a stack indicates the relative frequency of that nucleotide at the specified position of the CArG box, which is summarized numerically below the logo. Nucleotides within each stack are ordered from most (top) to least (bottom) frequent. Thus the consensus CArG depicted here is CCTTATATGG, which is close to the most frequent CArG element in this collection (CCTTTATGG, $n = 10$). Note that only 140 permutations of CArG (out of a theoretical 1216) are embodied in this sequence logo. Further details about CArG boxes, including the determination of the theoretical number of 1216, are reviewed elsewhere.^{5,27,107}

myocardin¹⁷ and MRTF-A¹⁸ compete with ELK1 for a common interface on SRF, thus allowing flexible SRF-dependent gene expression in varying contexts.

SRF binds as a dimer to at least 1216 permutations of a 10-bp segment of DNA known as the CArG box (Figure 1). CArG boxes are found mainly in the proximal promoter and the first intron of hundreds of experimentally validated or hypothesized target genes.^{19–26} Bioinformatic and wet-lab assays have disclosed a disproportionate number of SRF-target genes encoding for elements of the actin cytoskeleton.^{20,22,23,26} Evidence for the role of SRF in cytoskeletal processes stems from genetic ablation studies using slug, fly, and worm where loss of SRF function leads to defective animal/cellular locomotion (reviewed by Miano *et al*²⁷). In fact, SRF is essential for life processes in every species where it has been inactivated, including mice where global loss of SRF leads to incomplete gastrulation and embryonic arrest before organogenesis commences.²⁸ The latter phenotype is characterized by the inability of the mesodermally fated cells to properly migrate during the dynamic stages of germ-layer formation.^{28,29}

The development of transgenic mouse lines carrying cell-specific promoters directing the expression of a bacteriophage topoisomerase gene, Cre recombinase, has advanced our understanding of the function of SRF in various organ systems. Three distinct floxed *Srf* mice have been used to cross with specific Cre mice. Two were designed for deleting exon-1³⁰ or exon-2,³¹ the latter mouse yielding a hypomorphic

allele upon Cre-mediated excision. The other floxed mouse was engineered for deletion of the 5' promoter and the first exon. Studies using this floxed *Srf* mouse showed no measurable SRF entity after Cre-mediated excision.^{32,33} In this review, I highlight the salient pathologies associated with the genetic loss of SRF in individual organ systems of the mouse. An up-to-date list of cell-specific SRF knockouts is provided in Table 1, and the readers are encouraged to consult the original literature for some of the nuances associated with each knockout. Human and experimental models of disease showing changes in SRF expression are also reviewed. Perspectives relating to commonalities in phenotypes where SRF is ablated as well as future directions are provided at the end.

SRF AND CARDIOVASCULAR SYSTEM DISEASE

Heart

The heart is the first organ to develop as the rapidly expanding embryo outstrips the diffusion limits of nutrient and gas exchange. SRF expression is first seen in the mouse cardiac crescent at embryonic day 7.75 (e7.75)³⁴ where cardiac progenitor cells migrate to the midline of the embryo to form a linear heart tube at ~e8.25 followed by cardiac looping and septation.³⁵ As the embryo turns on its axis, the heart has already formed chambers and is contracting with abundant expression of SRF in the proliferating compact zone and finger-like projections called trabeculations that extend into the interior of the heart (Figure 2a). The most obvious phenotype in mice with cardiac muscle-specific inactivation of SRF is a thin-walled compact zone and poorly developed trabeculations of the ventricular chambers resulting in a dilated, heart failure-like phenotype with embryo demise occurring at ~e11.5 of development^{31,32,36,37} (Figure 2b). Wild-type embryonic cardiomyocytes have few bands of sarcomeres that somehow are sufficient to coordinate enough force to propel blood throughout the embryo. In SRF mutants, cardiomyocytes show severe disruption in cardiac sarcomerogenesis, which likely contributes to the heart failure phenotype.^{32,37} A similar ultrastructure phenotype is manifest in adult mouse hearts where SRF is inducibly inactivated using a tamoxifen-responsive Cre recombinase.³⁸ A mosaic knockout of SRF using the *Desmin* promoter driving Cre resulted in mutant cardiomyocytes in close apposition to wild-type cardiomyocytes. Despite the presence of some 50% SRF-positive myocytes, mosaic SRF-knockout mice showed increases in both interstitial fibrosis and heart weight to body weight, and eventually succumbed to heart failure by 11 months of age.³⁹ Not surprisingly, biochemical data from knockout mice show dramatic decreases in an array of SRF-target genes encoding for contractile elements and calcium-handling proteins.^{31,32,36,37,39} Reduced expression of such genes explains the inability of mutant hearts to organize functional sarcomeres and generate sufficient contractile force. As SRF activates a growing number of actin cytoskeletal genes important in cell migration,²⁷ the poorly

Table 1 Cell-specific SRF-knockout phenotypes

Cre driver ^a	Targeted cell type	Phenotype	Ref.
<i>Myh7</i> (β Mhc)	Cardiomyocyte	Dilated heart and embryo demise at e10.5–e13.5	31
<i>Tagln</i> (<i>Sm22α</i>)	Cardiomyocyte/SMC	Dilated heart/reduced SMC recruitment and embryo demise at e10.5	32
<i>Myh6</i> (α Mhc)	Cardiomyocyte	Cardiac insufficiency/reduced myocyte survival	36
<i>Nkx2.5</i>	Cardiomyocyte	Non-beating heart tube at e8.25	37
<i>Myh6</i> -MerCreMer	Adult cardiomyocytes	Heart failure and death 10 weeks after KO	38
<i>Des</i> (<i>Desmin</i>)	Cardiomyocyte	Heart failure and death by 11 months of age	39
<i>Tie1</i>	Endothelial cell	Aneurysm, hemorrhage, EC junctional/actin cytoskeletal defects, with death at e14.5	48
<i>Tie2</i>	Endothelial cell	Hemorrhage and EC junctional/actin cytoskeletal defects, with death at e14.5	49
<i>Myog</i> - <i>Mef2c</i>	Skeletal muscle cell	Perinatal orthopnea with thinned myofibers	61
<i>ACTA1</i> (<i>human α-skeletal actin</i>)	Adult skeletal muscle	Muscle hypotrophy and poor regeneration, with 50% mortality by 6 weeks of age	63
Tamoxifen-inducible <i>ACTA1</i>	Adult skeletal muscle	Muscle atrophy (sarcopenia), defective regeneration and accelerated muscle aging	64
<i>Alb</i> - <i>Afp</i> (<i>Albumin, α-fetoprotein</i>)	Hepatocyte	Reduced body size and chronic liver regeneration	67
<i>Alb</i> - <i>Afp</i>	Hepatocyte	Impaired hepatic regeneration	68
<i>Pdx1</i> (<i>pancreatic/duodenal homeobox-1</i>)	All pancreatic cells	Pancreatitis, loss of exocrine pancreas	69
Tamoxifen-inducible <i>Tagln</i> (<i>Sm22α</i>)	Adult SMC	Chronic intestinal pseudo-obstruction and death by 3 weeks after deletion of SRF	70,71
<i>Camk2a</i> (<i>Camkl1α</i>)	Forebrain neurons	Defective neuronal migration to hippocampus, impaired actin dynamics and axonal guidance	76,77
<i>Camk2a</i> or <i>Syn1</i> (<i>synapsin-1</i>)	Forebrain neurons	Attenuated activity-dependent immediate-early gene expression and long-term synaptic potentiation	33
<i>Camk2a</i>	Adult forebrain neurons	Impaired short-term memory and long-term depression	82
<i>Camk2a</i>	Hippocampal neurons	Defective layering of neurons and dendritic branching	79
<i>Wnt1</i>	Dorsal root ganglion	Defective extension/branching of axons	87
<i>Krt5</i> (<i>keratin-5</i>)	Keratinocytes	Embryonic lethality, skin hemorrhaging/blistering	88
RU486-inducible <i>Krt14</i> (<i>keratin-14</i>)	Keratinocytes	Psoriasis-like condition with epidermal hyperproliferation, cytoskeletal and cell–cell contact perturbations	88
<i>Krt14</i>	Keratinocytes	Thickened epidermis, loss of barrier function and cell–cell contacts due to abnormal cytoskeleton	89
<i>Cd4</i> (<i>cluster of differentiation-4</i>)	T lymphocytes	Loss in circulating T lymphocytes	90
<i>Cd19</i> (<i>cluster of differentiation-19</i>)	B lymphocytes	Decrease in marginal zone B cells, low IgM	90
<i>Mx1</i> (<i>Myxovirus resistance 1</i>)	Megakaryocyte	Thrombocytopenia and macrothrombocytopenia	91
<i>Pf4</i> (<i>Platelet factor 4</i>)	Megakaryocyte	Thrombocytopenia and macrothrombocytopenia; prolonged bleeding time	92

^aThe gene symbols indicate the promoters used to control the expression of Cre recombinase, with common gene aliases indicated in parentheses.

developed trabeculations essential for coordinated cardiac contraction may be a consequence of inefficient migration of cells from the compact zone. It will be informative to assess the embryonic cardiac function in SRF-null mice by ultrasound to further document the physiological consequences of the structural changes within the developing myocardium.

Transcription factors (eg, GATA4) are reduced in SRF-null hearts even though no functional SRF-binding CARG boxes have been identified in their promoter regions.^{31,32,36,37} This would support an indirect mechanism involving down-regulated expression of SRF-dependent transcription factors that normally activate these genes, or upregulation of

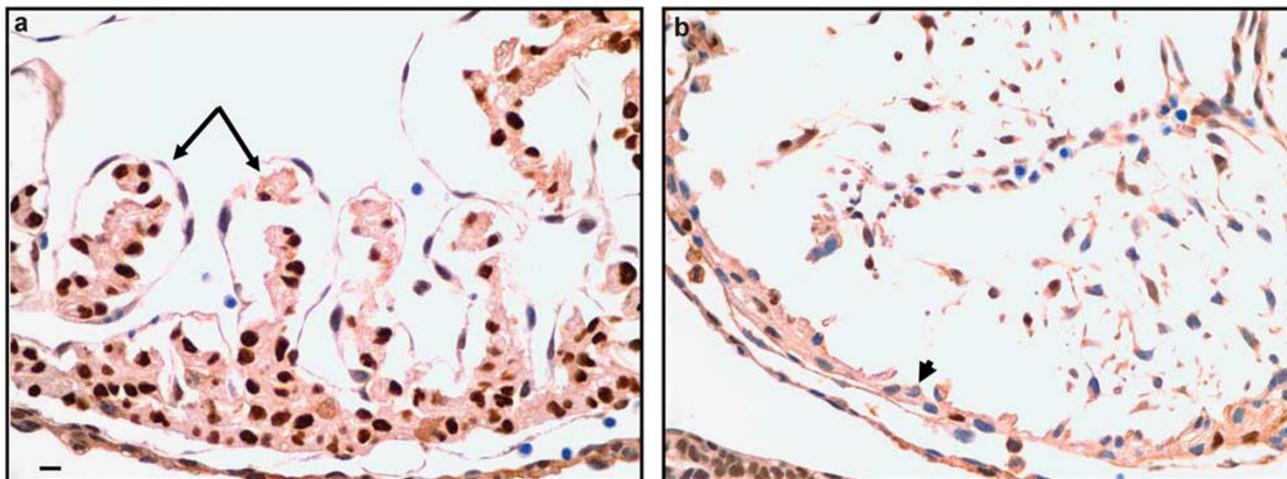


Figure 2 Expression of SRF in the developing myocardium. (a) A section of an e10.5 wild-type mouse heart stained for SRF (brown) in cardiomyocytes of the compact zone and finger-like extensions known as trabeculations projecting from this zone (arrows). (b) An e10.5 SRF-mutant heart showing reduced staining for SRF, a thinned compact zone (arrow head), and absence of trabeculations. The small scale bar at the lower left in panel a, 10 μ m.

repressive miRs. Interestingly, loss of expression of SRF in cardiomyocytes can activate a subset of genes.^{23,37} Although SRF is not considered to be a direct repressor of gene expression, it may indirectly silence gene activity through its ability to bind to and activate CARG-containing miRs.^{25,40,41} In fact, some upregulated genes in SRF-null cardiomyocytes have been shown to increase because of the loss in the expression of SRF-dependent miRs that normally limit such gene expression.³⁷

Sustained elevated levels of SRF in a single transgenic mouse line carrying human SRF under control of the *Myh6* promoter resulted in cardiomyopathy characterized by four-chamber dilatation, myocyte hypertrophy, interstitial fibrosis, and mitochondrial/myofiber damage.⁴² A subsequent report showed that lower SRF expression induced a milder phenotype with evidence of accelerated cardiac aging in young transgenic mice.⁴³ Both studies concluded that overexpression of SRF elicits a heart failure phenotype. In this context, human heart failure was reported to show elevations of a natural dominant-negative form of SRF arising from alternative splicing.⁴⁴ The dominant-negative SRF isoform potentially inhibited SRF-dependent gene expression, mirroring the biochemical phenotype seen in SRF-null mice.⁴⁴ A subsequent human heart failure study showed decreases in full-length SRF and elevated expression of a caspase-3-mediated cleaved product of SRF.⁴⁵ Similar to the natural dominant-negative SRF reported earlier,⁴⁴ this cleaved SRF product could inhibit the transcriptional activity of an SRF-target gene. Collectively, these experimental and clinical data indicate that levels of SRF must be strictly controlled to maintain cardiac homeostasis.

Blood Vessels

Blood vessel formation occurs initially in the yolk sac and in the primary plexi of the early embryo. The first vascular cell

to emerge is the endothelial cell (EC) whose tight intercellular connections are vital for the establishment of a closed circulatory system.^{46,47} EC-specific inactivation of SRF with a *Tie1*-Cre mouse resulted in embryonic vascular aneurysms and hemorrhaging of the forebrain and limb buds beginning at e11.5 of development; there was no such phenotype in the blood vessels of the yolk sac vasculature.⁴⁸ By contrast, *Tie2*-Cre-mediated loss of EC SRF led to disruptions in the embryonic and yolk sac blood vessels.⁴⁹ Despite differences in phenotype, both EC-specific SRF knockouts resulted in embryonic death at e14.5 of development. Moreover, both knockouts reported a disruption in inter-EC junctional complexes with reduced expression of principal junctional proteins such as E-cadherin⁴⁹ and vascular endothelial (VE) cadherin, and zona occludens.⁴⁸ Importantly, the mouse VE-cadherin promoter was shown to contain functional CARG elements that bind to SRF in a ChIP assay and direct CARG-dependent promoter activity *in vitro*.⁴⁸ Whether other EC intercellular complex genes are direct targets of SRF remains an open question. Another phenotype associated with loss of SRF in ECs was the disruption of the actin cytoskeleton in the so-called tip cells,⁴⁸ which give rise to sprouting ECs during angiogenesis.⁴⁷ This resulted in defective angiogenic sprouting and expansion of subjacent stalk cells, which could underlie the aneurysm phenotype.⁴⁸ It will be informative to delete SRF in adult ECs to further illuminate the role of this transcription factor in postnatal angiogenic responses.

The other major cell type in the vessel wall is the smooth muscle cell (SMC), which emerges from progenitor cells localized in distinct regions throughout the developing embryo.⁵⁰ Expression of SRF is already evident in vascular SMCs at \sim e10.5 of development,³⁴ a time coinciding with the expression of several SMC contractile genes^{51–54} and the establishment of a functional circulatory system.⁵⁵ The only reported knockout of SRF in vascular SMCs used the *Sm22 α*

promoter,³² which is active in embryonic SMCs, the developing heart, and somites.⁵⁶ The heart phenotype is very similar to that reported by others (Table 1). Inactivation of SRF in embryonic SMCs leads to a significant reduction in the number of peri-vascular progenitor cells that are fated to become aortic SMCs as well as defects in the cyto-architecture at e10.5 of development.³² A similar flawed actin cytoskeleton is seen in human adult coronary artery SMCs where SRF is knocked down.²⁷ Recently, siRNA-mediated knock-down of SRF in human coronary artery SMCs resulted in decreases in both SMC migration and proliferation.⁵⁷ Thus, the decrease in SMC investment of the embryonic aorta where SRF has been deleted could be a consequence of reduced growth, migration, or a combination of both processes.

Whether loss of SRF exclusively in vascular SMCs during development and postnatal life has consequences for normal vascular function awaits the use of more specific or inducible promoters that limit the deletion of SRF to only SMC lineages. For example, Cre recombinase was knocked into the endogenous *Sm22 α* gene and the mouse line showed only adult SMC-restricted activity; no activity was seen in the embryonic heart or blood vessels.⁵⁸ This Cre driver therefore represents a valuable tool to circumvent the embryonic lethality of knocking out SRF with a conventional *Sm22 α* promoter-driven Cre.³² Moreover, a tamoxifen-inducible, smooth-muscle myosin heavy-chain promoter-driven Cre will be of utility to inactivate SRF at any time during embryonic or postnatal development.⁵⁹

SRF AND SKELETAL MUSCLE SYSTEM DISEASE

The rostral-caudal segmentation of the paraxial mesoderm leads to the formation of somites where a subset of precursor cells are fated to become skeletal muscle.⁶⁰ Although *Sm22 α* is active during early somitogenesis, there was no reported embryonic phenotype in SRF-null somites, probably because of the early manifestation of cardiac/SMC defects.³² Using a hybrid *myogenin-Mef2c* promoter/enhancer driving Cre-mediated excision of SRF in early embryonic skeletal muscle precursors, Olson and co-workers showed perinatal death due to muscle fiber thinning and orthopnea.⁶¹ This phenotype is reminiscent of the myogenin knockout where mutant mice were born normally only to die at birth from an inability to breathe.⁶² Loss of SRF in the skeletal muscle of newborn mice showed structural and biochemical phenotypes similar to loss of SRF in the other two muscle types, namely pronounced disruption of muscle filament array and decreases in contractile gene expression.⁶¹ Knockout of SRF in post-mitotic skeletal muscle resulted in decreases in muscle mass (hypotrophy) and poorly organized sarcomeres, with only 50% of mutant mice surviving up to 6 weeks of age.⁶³ Inducible deletion of SRF in adult skeletal muscle with a tamoxifen-inducible Cre driver showed an apparent acceleration in atrophy seen commonly in aged mammals, including humans.⁶⁴ Adult skeletal muscle lacking SRF also showed a switch in muscle fiber type from fast/glycolytic to

slow/oxidative myofibers.^{63,64} All skeletal muscle-specific knockouts of SRF show muscle precursor cells that are fated to become skeletal muscle, but the maintenance of a normal contractile cell is lost due to muscle fiber atrophy. In addition, the capacity to regenerate skeletal muscle mass after injury, or during the normal aging process, is severely compromised in mutant mice.^{63,64} In this context, two putative SRF-target genes considered important for skeletal muscle's regenerative potential, *Igf1* and *Il4*, were shown to be downregulated with loss in SRF and both genes' promoters bind to SRF in a ChIP assay.⁶³ It remains to be formally demonstrated whether the CARG elements in the *Igf1*- and *Il4*- regulatory regions are functional using other assays (eg, luciferase).

Sarcopenia is a human condition of skeletal muscle wasting with attending loss in strength and generalized weakness that occurs in the aged population. Evidence suggests an age-dependent decline in SRF expression in humans.⁶⁴ Mice also show decreasing levels of SRF with advancing age.⁶⁵ One of the cofactors for SRF activity, MRTF-A, was also reduced in aged skeletal muscle.⁶⁵ Interestingly, transgenic mice overexpressing a dominant-negative form of MRTF-A show skeletal muscle atrophy.⁶¹ Finally, skeletal muscle from a spontaneous mouse mutant (merosin, *dy* mice), which is a model for muscular dystrophy, showed attenuated SRF expression.⁶⁶ These mice also showed a coincident increase in the expression of the negative skeletal muscle regulator, myostatin, although the mechanisms for this upregulation are unclear. Together, the available data indicate an important role for normal SRF levels in the maintenance of skeletal muscle function and its response to injury.

SRF AND DIGESTIVE SYSTEM DISEASE

Liver

The liver is a vital organ for detoxification of xenobiotics, glucose regulation, and metabolism of fats to produce and distribute triglycerides and cholesterol in the form of lipoproteins. The liver is also one of the most highly regenerative organs in the body. Murine hepatogenesis begins around e10.5 of development with detectable SRF in association with the hepatocyte-restricted transcription factor, *Hnf4a*.⁶⁷ Deletion of SRF specifically in hepatocytes was achieved by two independent groups using the *albumin* promoter linked to an α -fetoprotein enhancer. In one report, mutant mice were born healthy and fertile with no gross abnormality in the liver, but there was impaired regenerative capacity after partial hepatectomy.⁶⁸ The other study reported an incompletely penetrant postnatal lethal phenotype predominantly manifest in males.⁶⁷ Male mice showed abnormally low levels of circulating glucose, triglycerides, and IGF1, and weighed significantly less than the control littermates. Furthermore, adult mutant livers were shown to be in a 'perpetual state of regeneration' with increases in both hepatocyte proliferation and apoptosis.⁶⁷ Finally, microarray studies showed decreases in the expression of genes involved with nutrient and drug

metabolism, especially members of the cytochrome-P450 system.⁶⁷ Of note, the role of SRF deletion in the cytoskeleton and secretory abilities of the hepatocytes was not evaluated in either study.

Pancreas

The pancreas is both an exocrine and an endocrine organ participating in local digestive and distal hormonal regulatory processes. Detectable levels of SRF are seen in all cell types of the pancreas during embryonic and postnatal development, including the acinar and ductal cells of the exocrine pancreas and the endocrine cells of the islets of Langerhans.⁶⁹ Loss of SRF appears to have no effect in the endocrine pancreas, with normal numbers of islets of Langerhans and normal circulating levels of insulin, which is released from the β -cells of the endocrine pancreas.⁶⁹ It will be informative to examine the actin cytoskeleton in the SRF-null endocrine pancreas to determine whether secretion of insulin and hormones from other endocrine cells occur independent of a normal cyto-architecture. The exocrine pancreas of SRF-mutant mice shows impaired proliferation of acinar cells at 4 weeks of age and an inflammatory response, likely perpetuated by elevated NF- κ B expression, with fibrosis by 2 months.⁶⁹ There is also a dramatic increase in serum amylase and lipase suggestive of pancreatitis. Strikingly, by 11 months of age, the exocrine pancreas of the SRF mutants is completely lost and replaced with adipose tissue surrounding the normal endocrine pancreas.⁶⁹ It is unclear what exactly signals adipogenesis in the SRF-mutant exocrine pancreas.

Gastrointestinal Tract

Using a tamoxifen-inducible Cre under the control of the *Sm22 α* promoter, two groups showed that loss of SRF in the intestinal tract results in a model for a human condition known as chronic intestinal pseudo-obstruction.^{70,71} This phenotype is characterized by loss of intestinal peristalsis due to defective SMC contractility. Predictably, inactivation of SRF resulted in attenuated levels of several SMC contractile genes in both the colon and the duodenum. Furthermore, the cyto-architecture of mutant colonic SMCs was completely in disarray as evidenced by a diminution in the F-actin-to-G-actin ratio. Mice eventually succumbed to cachexia, dehydration, and death \sim 3 weeks after the onset of SRF depletion.

Gastric ulceration in both humans and experimental models stimulates increases in SRF expression within the cells of the lamina propria and the evolving granulation tissue.⁷² Similarly, SRF level is elevated in an experimental model of esophageal ulceration.⁷³ Overexpression of SRF in a rat model of gastric/esophageal ulceration caused an amelioration of the condition with increased re-epithelialization and submucosal SMC regeneration.^{72,73} This healing of the ulcer appears to be a function of the activation of growth-related genes by SRF and subsequent proliferation of epithelial cells, SMCs, and myofibroblasts. Conversely, reductions of SRF in experimental models of ulceration, using antisense RNA,

resulted in impaired angiogenesis of the microvasculature, presumably because of defective vascular endothelial growth factor-dependent signaling.⁷⁴ These results suggest that SRF could be a therapeutic target in the setting of gastro-esophageal ulceration.

SRF AND NERVOUS SYSTEM DISEASE

Central Nervous System

Normal CNS development requires carefully orchestrated cell-migratory events to achieve the proper neuronal landscape for synaptic transmission and homeostasis in cognition, learning, memory, and motor acquisition. Expression of the SRF protein has been documented in a variety of CNS neurons during early postnatal development, most notably in the neurons of the hippocampal dentate gyrus and the cortex.⁷⁵ Deletion of SRF in prenatal forebrain neurons resulted in an accumulation of precursor neurons at the sub-ventricular zone (SVZ) and a hypoplastic hippocampus leading to disturbances in eating such that animals died by postnatal day 21.⁷⁶ *In vitro* assays showed impaired neuronal precursor migration from the SVZ, which likely explains the observed accumulation of precursor cells at the SVZ *in vivo*. Cells of the dentate gyrus showed a reduction in F-actin, and biochemical data indicated decreases in gelsolin and altered phosphorylation of cofilin, two regulators of cytoskeletal dynamics. While both cofilin genes are direct targets of SRF,²² the nature of SRF-dependent gelsolin expression is incompletely understood. SRF deletion in the perinatal hippocampus resulted in poor organization and aberrant synapses of the so-called mossy fibers, apparently because of their inability to respond appropriately to guidance cues that drive proper neuronal cell migration and organization.⁷⁷ Furthermore, cultured SRF-null neurons showed decreases in axonal length, neurite outgrowth, and actin dynamics.⁷⁷ Microarray analysis of SRF-null hippocampi showed reduced expression of a large number of oligodendrocyte-associated genes, including those essential for myelination of neurons.⁷⁸ Further analysis showed a non-cell-autonomous increase in oligodendrocyte precursors and reduced differentiated oligodendrocytes in SRF mutants, with reductions in various myelin-related genes.⁷⁸ Thus loss of neuronal SRF has severe consequences for the normal development of at least one glial cell type. Recently, loss of SRF in the CNS of perinatal pups resulted in disorganized layering of both hippocampal and cortical neurons with defective dendritic branching and spine formation.⁷⁹ These phenotypes bear some resemblance to those reported in mice carrying null alleles for the components of reelin signaling.⁸⁰ Interestingly, reelin directs the expression of immediate-early genes and the elements of the actin cytoskeleton showing a functional axis connecting reelin signaling to SRF-dependent gene expression.⁷⁹

In contrast to the initial report of SRF deletion in CNS neurons,⁷⁶ an independent group using the same *Camk2a* Cre driver did not report any defects in the neuronal cyto-architecture and migration or postnatal lethality.³³

Instead, they showed decreases in SRF-dependent immediate-early gene expression (eg, *Fos*) after exposure of mice to a new environment and impaired long-term potentiation of cortical neurons suggestive of synaptic pathology. Of note, SRF expression and binding activity to a CARG box have been shown to increase in a rat model of epilepsy,⁸¹ a disease of neuronal hyper-excitability and neuronal plasticity, which affects up to 2% of the world population. Whether loss of SRF expression has any consequences for the pathology associated with epilepsy, especially more chronic forms of the disease, is an interesting question for future research. Further evaluation of a forebrain SRF-knockout mouse model showed no change in the neuronal number; however, behavioral studies showed deficits in habituation to a novel spatial stimulus and defective memory and learning.⁸² Microarray profiling of the hippocampi from this SRF-knockout mouse showed attenuated expression of the genes involved in the release of calcium (eg, ryanodine receptors 1 and 3), although again, it is unclear whether these are direct or indirect SRF-target genes.⁸²

Alzheimer's disease (AD) is the most common form of dementia and represents, arguably, one of the most challenging ailments confronting the World Health Organization as no effective treatment exists to prevent or slow disease progression. Furthermore, AD is very difficult to accurately diagnose in patients, and there is an expected sharp increase in the number of people who will acquire AD. Historically, the pathogenesis of AD centered on neuronal degeneration and loss; however, a fundamental problem of the cerebral vasculature appears to mediate the characteristic loss of neuronal homeostasis and synaptic transmission leading to cognitive decline. Indeed, two cardinal features of AD pathology are reduced blood flow to the brain parenchyma (hypoperfusion) and accumulation of amyloid β -peptides both in the brain itself and around blood vessels.^{83,84} Interestingly, SRF is increased in SMCs of cerebral blood vessels taken from human AD patients, and the elevation in SRF appears to mediate, in part, cerebral hypo-perfusion as reducing SRF with a short-hairpin RNA normalized contractile activity and cerebral blood flow in experimental animals.⁸⁵ Furthermore, increases in SRF were recently shown to impede the normal clearance of amyloid β -peptides through direct activation of *SREBP2*, a known repressor of the major amyloid β clearance receptor, *LRP1*.⁸⁶ Thus, SRF has emerged as a putative target for therapeutic intervention of this devastating disease that is estimated to afflict some 30 million people worldwide.

Peripheral Nervous System

The onset of SRF expression in the sensory neurons of the mouse dorsal root ganglion (DRG) occurs between e11.5 and e13.5 of development.⁸⁷ Using a *Wnt1*-Cre driver, which is active in the neural crest-derived progenitors of the DRG, Ginty and co-workers reported late embryonic lethality in SRF mutants due to a vascular patterning defect.⁸⁷ An ex-

amination of DRG neurons at e17.5 showed a normal complement of cells indicating SRF was dispensable for neuronal survival. However, there were perturbations in axonal extension, axonal branching, and target innervation similar to reports regarding CNS neurons lacking SRF (see above). As with CNS neurons, DRG neurons appear to require SRF-dependent cytoskeletal gene expression for proper outgrowth and branching.⁸⁷ It will be informative to ascertain the role of SRF in DRG regeneration after spinal cord injury during adult life. In addition, there are no reports of SRF functions within the neurons of the cerebellum where SRF expression has been shown.⁷⁵

SRF AND INTEGUMENTARY SYSTEM DISEASE

The epidermis functions as a crucial barrier between the internal body and the external world of microorganisms that otherwise would invade and colonize the body's interior. The epidermis is composed of stratified epithelium, which first emerges in the mouse embryo between e13.5 and e15.5 of development. Deletion of SRF in the keratinocytes of the epidermis with a keratin-5 promoter-driven Cre resulted in embryonic lethality \sim e16.5 with severe blistering, edema, and hemorrhaging of skin.⁸⁸ When an RU486-inducible keratin-14 (*Krt14*) Cre mouse was used to inactivate SRF in keratinocytes, mice were born alive but spontaneously developed hyper-proliferative skin lesions and inflammation.⁸⁸ An independent study reported the inactivation of SRF with a non-inducible *Krt14*-Cre and showed perinatal death with a compromise in normal barrier function, but no evidence for hyper-proliferation of the thickened epidermis.⁸⁹ Both studies reported decreases in cell-matrix and/or cell-cell junctional contacts, and improper adhesion and differentiation of keratinocytes, apparently stemming from a defective actin cytoskeleton. These cell-cell alterations are similar to those reported in ECs lacking SRF (see above).

Human psoriasis is a hyper-proliferative disease of the epidermis and is associated with a decrease in SRF expression, a finding consistent with one of the phenotypes reported with loss of SRF expression in keratinocytes.⁸⁸ Conversely, a spontaneous mouse mutant (*corn1*) showing hyper-proliferation of corneal epithelial cells, showed increases in SRF expression and many of its target genes, including several keratin isoforms.²⁶ Further evaluation of these mice showed changes in actin dynamics leading to an augmented F-actin cytoskeleton. Whether this mouse model reflects the pathogenic changes associated with human cataracts is an interesting question for future investigation. Collectively, loss- and gain-of-function studies clearly indicate a vital role for SRF in establishing a tightly associated, differentiated surface epidermis that must function to provide a barrier to the underlying tissues of the body.

SRF AND HEMATOPOIETIC SYSTEM DISEASE

In the event that a breach occurs in the epidermis of skin, the body relies heavily upon the circulating and resident immune

cells to combat foreign antigens or pathogenic microorganisms. There is a paucity of information regarding the role of SRF in immune cell function. However, one study used a T-cell-specific Cre (*Cd4*) or a B-cell-specific Cre (*Cd19*) to selectively delete SRF in each respective population of lymphocytes.⁹⁰ The results showed that SRF was essential for T-cell maturation as few peripheral T cells were detected in SRF-null mice. Conversely, B-cell-specific deletion of SRF resulted in a mild phenotype characterized by normal B2-type B cells but a reduction in the number of marginal zone B cells. There was also a measurable decrease in the B-cell-surface expression of IgM from the marrow and spleen. Microarray experiments showed an expected decrease in the level of several SRF-target genes, including *Fos* and actin-associated genes, although the consequence for loss in such gene expression in this context is unclear.⁹⁰

In two very recent reports, SRF was inactivated in megakaryocytes using either an inducible Mx1-Cre driver⁹¹ or platelet factor 4 Cre.⁹² Mice with deficient levels of SRF in megakaryocytes exhibit thrombocytopenia and macrothrombocytopenia with reductions in numerous cytoskeletal-associated genes. The expected reduction in actin cytoskeletal genes likely explains the decrease in the number of proplatelets budding from megakaryocytes. Interestingly, the number of megakaryocytes lacking SRF accumulated in bone marrow and spleen. Moreover, defective platelet number and cytoarchitecture were accompanied by a prolongation in bleeding time.⁹² Taken together, these findings clearly establish a role for SRF in normal platelet production and function. It will be of interest to determine whether the reduction in platelets with loss in SRF has consequences for vascular remodeling following arterial injury or other platelet-associated disorders.

Several viral genomes have been shown to harbor functional CARG boxes that bind to SRF, suggesting a role for SRF in the pathogenesis of certain viral diseases.⁹³ Interestingly, a CARG box in the human T-cell leukemia virus-1 that deviates from the conventional sequences, shown in Figure 1, was shown to bind to and be activated by SRF.⁹⁴ This would suggest that the full complement of SRF-binding sites in the genome (ie, CARGome²²) may be more diverse than previously thought. Indeed, several putative SRF-binding 'CARG boxes' derived from genome-wide binding studies show sequences that diverge substantially from conventional CARG boxes.^{21,24} However, all such putative SRF-target sequences must be validated in a rigorous manner before they are formally considered part of the functional CARGome.

SRF AND PULMONARY SYSTEM DISEASE

The lung is endowed with numerous diverse cell types, yet there have been no published SRF knockouts in this organ. Elevated levels of SRF have been reported in lymphangioliomyomatosis (LAM), a rare disorder of interstitial pulmonary SMCs.⁹⁵ Induced SRF expression resulted in the upregulation of two matrix metalloproteinases (2 and 14)

and a reduction in one of the inhibitors of metalloproteinases (TIMP3), all of which are characteristic of LAM.⁹⁵ As with other putative targets, whether these effects are directed through SRF-binding CARG elements is unknown. In hypoplastic lungs of humans, where a stretch signal known to promote alternate SRF splice variants favoring normal pulmonary myogenesis is thought to be missing, the levels of full-length SRF are reduced.⁹⁶ Finally, in a mouse model of pulmonary interstitial fibrosis, where activated myofibroblasts contribute to pathology, the levels of SRF increase and presumably activate genes favoring the fibrotic phenotype in this model.⁹⁷ Future work should be directed toward the development of SRF-knockout models using established pulmonary epithelial cell-restricted Cre drivers such as *Ccsp*,⁹⁸ and toward assessing SRF expression and activity in asthma, pulmonary hypertension, and chronic obstructive pulmonary disease.

SRF AND CANCER

SRF was shown initially to bind to and transactivate the *Fos* promoter, suggesting a role in growth-related processes.⁶ Several studies have shown a correlation between human cancer and elevated levels of SRF or SRF binding to CARG boxes.^{99–102} Whether such increases in SRF are causative or merely reflective of human cancer is unclear. Recently, however, the liver-restricted miR-122 was shown to be attenuated in human hepatocellular carcinomas where SRF levels are elevated.¹⁰³ If miR-122 levels were reconstituted in cancer cells, tumorigenesis was reduced. Further studies showed that miR-122 targets SRF for degradation through a 3' untranslated region (UTR)-binding site; when SRF (lacking its 3' UTR) was introduced into miR-122-expressing cells, the growth-inhibitory action of miR-122 was partially lost, suggesting a direct role for SRF in a cancer phenotype.¹⁰³ Related RNA interference studies targeting SRF or MRTF-A reduced the metastatic potential of cancer cells by blocking target genes important in cell spreading, adhesion, and motility.¹⁰⁴ These results provide a basis for targeting SRF for the potential treatment of cancer. Interestingly, CARG elements are sensitive to ionizing radiation, making them potentially efficacious for radiation-induced gene therapeutic approaches.¹⁰⁵

PERSPECTIVE AND FUTURE DIRECTIONS

The advent of transgenic mice carrying various cell-specific promoters driving Cre recombinase has allowed the tissue-restricted inactivation of SRF across organ systems and the development of new models of human disease. Furthermore, a growing number of human diseases show alterations in the expression of SRF and its target genes, thus disturbing normal homeostatic processes. A common thread among both loss-of-function gene knockout studies of SRF and gain-of-function transgenic mice or human pathological conditions is the crucial role of SRF in establishing and maintaining a normal actin cytoskeleton. Many of the

phenotypes described in this review relate to aberrant cell migration (eg, vascular SMCs, neuronal cells, cancer cells) that results in defective organogenesis and/or abnormal function. Cell migration requires a series of coordinated steps requiring multiple components of the actin cytoskeleton, a growing number of which are direct transcriptional targets of SRF.²² It will be important to sort out which cofactor is used by SRF to stimulate these and other SRF-target genes. In addition, there may be subsets of SRF-dependent miRs that fine-tune the composition of the actin cytoskeleton. Identification of such molecular signatures could prove beneficial in directing therapies to a variety of diseases where altered cell migration is manifest.

As summarized in Table 1, almost every organ system relies on SRF for normal development and function. Future work should exploit other inducible or cell-specific Cre drivers to inactivate SRF in such cells as osteoblasts, osteoclasts, macrophages, and epithelial cells of the pulmonary tree. Another goal will be to gain a better understanding of the signaling and (post)transcriptional pathways linked to altered SRF expression, especially in human diseases. In addition, one could envisage the development of novel therapies that target the ability of SRF to interact with cell-specific co-regulators. For example, uncoupling the interaction between SRF and myocardin could find utility in the treatment of AD. Note that targeting SRF, *per se*, may not be ideal as changes in its expression or its ability to make stable contacts with CARG boxes would likely disrupt a broader number of target genes, unless targeting could be limited to the affected tissue. In this context, full disclosure of the functional CARGome is crucial to understanding the full complement of SRF-target genes (especially miRs) across cell types. Some SRF-dependent genes may emerge as viable targets of therapy to combat certain diseases.

Finally, there are three non-validated frameshift mutations within the coding region of human SRF, all of which create premature stop codons resulting in C-terminal truncations of SRF that would not be functional. Conversely, 14 SNPs exist in the 3' UTR of SRF, three of which have been validated. Whether the validated 3'-UTR SNPs alter miR binding should be a question for future study. We have uncovered SNPs within or adjacent to several human CARG boxes, and in two cases the SNP reduced SRF binding (submitted). Functional SNPs in SRF/CARG or in any of the co-regulators of SRF should be catalogued for future association studies that may implicate such allelic variants in human disease.

DISCLOSURE/CONFLICT OF INTEREST

The author owns shares in a start-up company, Socratech, whose charge is to develop novel therapies for neurodegenerative disorders.

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