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Review

Multi-phenotypic Role of Serum Response Factor in the Gastrointestinal System

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Serum response factor (SRF) is a master transcription factor of the actin cytoskeleton that binds to highly conserved CArG boxes located within the majority of smooth muscle cell (SMC)-restricted promoters/enhancers. Although most studies of SRF focus on skeletal muscle, cardiac muscle, and vascular SMCs, SRF research has recently expanded into the gastrointestinal (GI) system. Genome scale analyses of GI SMC transcriptome and CArG boxes (CArGome) have identified new SRF target genes. In addition to circular and longitudinal smooth muscle layers, SRF is also expressed in GI mucosa and cancers. In the GI tract, SRF is the central regulator of genes involved in apoptosis, dedifferentiation, proliferation, and migration of cells. Since SRF is the cell phenotypic modulator, it may play an essential role in the development of myopathy, hypertrophy, ulcers, gastric and colon cancers within the GI tract. Given the multi-functional role displayed by SRF in the digestive system, SRF has received more attention emerging as a potential therapeutic target. This review summarizes the findings in SRF research pertaining to the GI tract and provides valuable insight into future directions.
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Key Words
Gastrointestinal diseases; Knockout; MicroRNAs; Myocytes, Smooth muscle; Serum response factor

Introduction

Serum response factor (SRF) was first identified by Treisman in 1986. He discovered that SRF transcriptionally activates the *c-fos* gene in susceptible cells after serum stimulation by binding a conserved DNA sequence having dyad symmetry (SRE, serum response element) located within the immediate promoter region.[1] The SRE contains a core 10-nucleotide [CC $(A/T)_6$ GG] sequence (now called a CArG box), which was subsequently identified in the promoter and intronic regions of most smooth muscle cell (SMC)-restricted genes.[2,3] SRF binds to CArG boxes and transcriptionally activates target genes through direct association with more than 60 cofactors.[4,5] The identification of functional CArG boxes in the genome (i.e., CArGome) has begun to be elucidated[6-9] though there are likely many more CArG-containing genes awaiting discovery. A smooth muscle genome and CArGome browser containing genome-wide CArG boxes alongside SMC transcriptome data has been built and available to search and identify new CArG-containing genes.[10]

In addition to SRF-dependent protein-coding contractile mRNA genes, the SRF was recognized as an important regulator of many microRNA (miRNA) genes.[11] Many SRF-induced miRNA genes have been identified and they appear to be abun-

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dantly and predominantly expressed in SMCs.[12-14] The mechanism of SRF regulation of miRNA genes is the same as mRNA genes: both use CArG boxes. Many highly expressed miRNA genes in SMCs are activated by SRF suggesting that SRF-dependent miRNAs largely drive the SMC phenotype.

SRF has a multi-functional role in regulating SMC growth, differentiation, and death in the gastrointestinal (GI) tract. The diverse roles of the protein were uncovered by use of transgenic *Srf* knockout studies using cell-specific Cre-lox systems. Loss of the functional *Srf* gene shows that SRF is required for cardiac and smooth muscle development both in embryos and maintenance in adults.[15-19]

Abnormal expression of SRF is common in several GI diseases. Normal expression of the protein is essential for GI SMC differentiation. Loss or reduction of SRF may trigger myopathy[12,19] hypertrophy of SMCs[18] or GI cancers,[20] while overexpression of the protein may be linked to ulcers.[21]

Smooth Muscle Cell Transcriptome

SMC transcriptomes were recently obtained from the jejunum and colon.[10] SMCs express 16 000 genes, which are transcribed into 55 000 transcriptional variants. The most highly expressed genes are related to muscular contraction. SMC contractility is regulated by Ca^{2+} via ion channels and transporters.[22,23] They express as many as 447 ion channel and transporter isoforms, indicating that SMC excitability is regulated by a complex coordinated effort of numerous ion channels and transporters.[10] Within the ion channel family, calcium channels were the most abundantly expressed in SMCs,[10] and the predominance of calcium channel expression is consistent with the current paradigm for excitation-contraction coupling, which is primarily regulated by Ca^{2+} via calcium channels.[22] Many highly expressed and SMC-restricted ion channel isoforms and regulators such as *Kcnmb1*, *Ryr3*, *Jph2*, and *Dmpk* appear to be regulated by SRF.[10,24]

CArGome and Serum Response Factor Binding Sites

Genome-wide SRF binding CArG boxes were mapped in mice[10] and humans.[6] A large number of CArG boxes (98 236) are conserved between the 2 species. In addition, over 1 000 genes are associated with SRF binding CArG boxes, most of which are found within the promoter region, exon 1 and/or intron 1.[10] A hundred SRF-associated genes are highly expressed in SMCs, most of which appear to be specific to the cells.[10]

Smooth Muscle Genome and CArGome Browser

Although many SRF-regulated genes were identified and validated, there are still many protein-coding and non-coding genes in the genome that need to be discovered. To facilitate the analysis of genes in relation to SRF binding sites and CArG boxes, we built an interactive SMC genome and CArGome browser for mice[10]: http://medicine.nevada.edu/physio/transcriptome (requires Google Chrome and takes 1-2 minutes to upload the large files). This genome browser offers a new perspective into the alternative expression of genes in the context of SRF binding sites in SMCs, thus providing a valuable reference for future functional studies. For example, Figure 1 shows 6 transcriptional variants expressed in jejunal and colonic SMCs, 2 CArG boxes, and 1 SRF binding

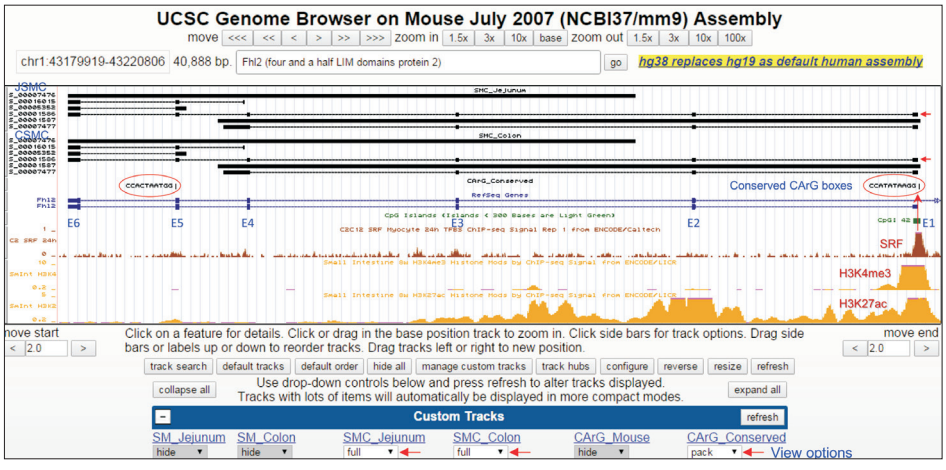

Figure 1. Transcriptional variants expressed in jejunal and colonic smooth muscle cells (SMCs), serum response factor (SRF) binding site, CArG boxes, CpG island, H3K4me3, and H3K27 activities of Fhl2 gene shown on the Smooth Muscle Genome and CArGome Browser.

site in an oncogene *Fhl2* on the browser. The SRF binding site in the proximal promoter region contains a conserved CArG box "CCATATAAGG" that overlaps with CpG island (DNA methylation sites), histone methylation H3K4me3 marks (active or poised gene), and histone acetylation H3K27ac marks (active gene).

Serum Response Factor Knockout Phenotype

Several animal studies have shown that SRF is a key regulator in the development and maintenance of both embryonic and adult muscle cells. All SRF deficient phenotypes generally have defects in the development and/or maintenance of the heart and GI tract. However, the phenotypes depend on when the gene is knocked out and which promoter drives the expression of *Cre* recombinase (Table 1). For example, in congenital knockout systems, *Srf* gene knockouts are embryonic lethal and exhibit the cardiac or GI smooth muscle defects.[16-19] However, embryonic survival varies between E10.5 and E18. This suggests that each promoter used is activated to knockout the gene at different time points. *Myh11*-driven knockout of *Srf* leads to extended survival of embryos (E18) as compared to the survival observed when *Srf* is inactivated with other promoters, including *SM22α*. Consistent with the congenital studies, inducible knockout of the gene in adult SMCs resulted in severe GI dilation with thinning of the smooth muscle layers.[12,25,26] In addition, *Myh11*-driven knockout mice survived longer than *SM22α*-driven knockouts. This phenotypic variation in 2 different promoters likely relates to the activation time and the strength of each promoter. Indeed, *SM22α-Cre* is activated earlier at E9.5[16,27] than *Myh11-Cre* at E13.5.[28] *SM22α* (*Tagln*) is expressed higher than *Myh11* in jejunal and colon SMCs.[10] Furthermore, *Myh11* and *SM22α* are differentially expressed in SMCs. *Myh11* is expressed exclusively in differentiated SMCs,[29] whereas *SM22α* is expressed in a less restrictive manner in proliferating as well as differentiated SMCs.[30] Nevertheless, the 2 phenotypes are similar in congenital and inducible knockout animals, suggesting that SRF is necessary for cardiac and smooth muscle development in embryos and maintenance in adults.

Serum Response Factor-induced MicroRNAs

miRNAs are required for the development and maintenance of GI SMCs. There are 2 important RNAse III enzymes in miRNA biogenesis, Dicer and Drosha that cleave primary transcripts to generate precursor miRNAs and mature miRNA duplexes respectively.[31,32] We demonstrated that GI SMCs could not survive without miRNAs in the RNAse III enzyme *Dicer* deficiency model.[33] The SMC-specific *Dicer* null mice developed severe dilation of the intestinal tract associated with the thinning and degeneration of the smooth muscle layers. A similar phenotype in SMC-specific *Drosha* null mice was observed although *Drosha* null mice showed a more severe pathological phenotype than *Dicer* null mice (unpublished data). This SMC degeneration also resembles that of SMC-specific *Srf* null mice, suggesting that SRF regulates expression of a large number of miRNAs in SMCs.[12] We previously found that GI smooth muscle of mice expressed 312 miRNAs, of which 36 were SRF-dependent as evidenced by in vitro *Srf* knock-down.[13] Further, using an advanced miRNA-seq technology, we identified 891 miRNAs from *Srf* wild type and deficient smooth muscle, of which 124 were induced by SRF.[12] SRF-induced miRNAs are highly expressed in GI SMCs as evidenced by the fact that over 95% of miRNAs were decreased in *Srf* deficient smooth muscle. The most highly expressed miRNAs in GI SMCs are miR-145 and miR-143 (account for 78% of all miRNAs) in which expression is SRF-dependent in in vitro knock-down and in vivo knockout systems.[12,13] In fact, miR-143 and miR-145 are generated from the same primary transcript, and binding of SRF to a conserved

Table 1. Phenotype of *Srf* Deficient Mice

Promoter	Specificity	Knockout	Phenotype	Survival
Srf[45]	Global	Congenital	Gastrulation defect	E9.5
Myh6[17]	Cardiac muscle	Congenital	Cardiac defect	E12.5
Myh7[19]	Cardiac muscle	Congenital	Cardiac defect	E10.5-13.5
SM22α[16]	Smooth muscle	Congenital	Cardiac and GI defects	E11.5
Myh11[18]	Smooth muscle	Congenital	Cardiac and GI defects	E18
SM22α[25,26]	Smooth muscle	Inducible	GI and bladder dilation	PT8-22
Myh11[12]	Smooth muscle	Inducible	GI dilation	PT21-28

E, embryonic day; GI, gastrointestinal; PT, post tamoxifen injection day.

CArG box located in the distal promoter region modulates their expression.[34] SRF-induced miR-143 and miR-145 expression promotes GI SMC differentiation and suppression of proliferation.[13] In addition, deficiency of *Dicer* in SMCs induces expression of genes involved in cell killing and death,[33] suggesting that the miRNAs may also suppress apoptotic genes. Taken together, miRNA studies indicate that SRF-induced miRNAs suppress proliferation and apoptosis of SMCs, and thereby promote differentiation of the cells in the GI tract. These miRNA regulatory pathways add to the complexity of SRF influence on epigenetic regulation of the GI SMC phenotype.

Apoptosis

Recent studies showed that SRF regulates apoptosis. We found that SMCs undergo massive apoptosis in the absence of SRF expression in a transgenic knockout mouse model.[12] In SMC-restricted *Srf* inducible knockout mice, SMC degeneration occurs by abnormally overexpressed apoptotic proteins in SRF-dependent and anti-apoptotic miRNA deficiency. This new role of SRF as an anti-apoptotic regulator is supported by recent findings (Table 2). Parlakian et al[19] first observed that cardiac-restricted *Srf* depletion induces caspase 3 and apoptosis in the embryonic heart. Wiese et al[35] recently reported that restoration of SRF antagonized Myc repression of SRF target genes, attenuated Myc-induced apoptosis, and reverted a Myc-dependent decrease in Akt phosphorylation and activity. Sisson et al[36] also recently confirmed that a SRF/MRTF pathway inhibitor CCG-203971 promotes myofibroblast apoptosis, decreases alveolar plasminogen activator inhibitor-1, and leads to significantly reduced lung collagen content, thereby decreasing lung fibrosis. Furthermore, Bae et al[37] showed that antisense inhibition of SRF expression in SH-J1 cells significantly enhanced the apoptotic effects of sorafenib, an oral multi-kinase inhibitor, while reducing expression of mesenchymal markers and restoring expression of E-cadherin. Chen et al[38] also recently reported that miR-320a contributed to atherogenesis by down-regulating SRF, inhibiting human-derived endothelium cell proliferation and inducing apoptosis. Lastly, Huang et al[39] reported that apoptotic activities (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling [TUNEL], caspase 3, caspase 9, p53, nuclear chromatin aggregation, nuclear fragmentation, and cytoplasmic apoptotic body formation) are increased in the aortic smooth muscle with conditional knockout of *Myocd*, which are strikingly similar to those of our DNA fragmentation, ultrastructural and TUNEL assay findings in the GI smooth muscle of *Srf* knockout mice. The remarkable similarities in the gross, microscopic, and molecular findings of *Myocd* knockout and *Srf* knockout mouse models are not surprising given that myocardin is an important transcriptional coactivator that binds directly to SRF to activate the transcription of a subset of SRF-regulated genes encoding cytoskeletal and contractile proteins.[40,41] All of the evidence above clearly indicates that SRF suppresses apoptotic activities in SRF-restricted cells including GI SMCs.

Hypertrophy

SMCs change their behavior in response to intestinal injuries. Under the hypertrophic condition of small bowel partial obstruc-

Table 2. Evidence of Serum Response Factor in Apoptosis

Model	Target gene	Genetic change	Phenotype
Congenital knockout of *Srf* in heart[19]	*Srf*	Caspase 3 ↑	Apoptosis in embryonic heart ↑
Conditional knockout of *Srf* in SMCs[12]	SRF-dependent miRNAs ↓	Apoptotic proteins ↑	SMC apoptosis ↑
Myc/Miz1 mediated SRF repression in epithelial cells[35]	SRF/MRTF target genes ↓	Akt phosphorylation and activity ↓	Myc-induced apoptosis ↑
SRF/MRTF pathway inhibitor in lung fibrosis[36]	SRF/MRTF target genes ↓	Alveolar plasminogen activator inhibitor-1 ↓ collagen ↓	Myofibroblast apoptosis ↑ fibrosis ↓
Antisense inhibition of SRF expression in SH-J1 cells[37]		E-cadherin ↑	Apoptotic effects of sorafenib ↑
miR-320a in atherogenesis[38]	*Srf*		Apoptosis ↑
Conditional knockout of *Myocd* in SMCs[39]	SRF/MYOCD target genes	Caspase 3, caspase 9 and p53 ↑	Apoptosis ↑

SRF, serum response factor; SMCs, smooth muscle cells.

tion, we recently found that SMCs are dedifferentiated into myofibroblast-like cells (platelet-derived growth factor receptor α [PDGFRα]^{low} cells) with low-level expression of SRF.[18] Consistently, *Srf* expression is dramatically reduced in dedifferentiated and growing PDGFRα^{+} cells in a cell culture condition.[18] The PDGFRα^{low} cells are highly proliferative and responsible for the thickness of the hypertrophied muscle.[18] The partial obstruction bowel model results in a transient hyperplasia at the beginning, followed by a prolonged hypertrophic response of intestinal SMCs.[42] We also found that cell proliferation is transiently increased in smooth muscle layers at the beginning of SMC-restricted, inducible *Srf* knockout in adult mice.[12] SMC proliferation is regulated by the myogenic repressor ELK1 bound to SRF.[43] Since ELK1 competes with another cofactor MYOCD for the same binding region of SRF, SMC phenotype depends on amount of these two antagonistic cofactors.[43] Chen et al[42] showed that the expression of *Elk1* is immediately and transiently induced within 6 hours in the partial obstruction model, but the expression level of the cofactor comes back to normal, and is not significantly changed during the development of hyperplasia and/or hypertrophy. If ELK1 is required for GI SMC hyperplasia, expression of the protein should be gradually increased to bind dominantly to SRF. We observed that expression of *Elk1* is decreased as SMCs become proliferative PDGFRα^{low} cells.[18] Chevigny et al[44] recently reported that expression of nuclear ELK1 is not changed in hyperplastic and hypertrophic airway smooth muscle in asthma. Further studies are obviously required to demonstrate if ELK1 regulates GI SMC proliferation. Nevertheless, the evidence above indicates that hyperplasia and hypertrophy develop in the GI smooth muscle when expression of SRF is reduced in SMCs.

Contractility

The role of SRF in SMC growth, differentiation, and phenotypic maintenance has been well established.[2,5,40] SRF regulates expression of most SMC-specific contractile and contractile-associated proteins, including smooth muscle myosin heavy chain, SM α-actin, SM22, and calponin, by binding to highly conserved CArG boxes that are located within the majority of SMC-restricted promoters/enhancers.[3] Lack or decrease of SRF is directly linked to a phenotypic change of SMCs, leading to hypomotility of smooth muscle in the GI tract. Deficiency of SRF in SMCs of *Srf* knockout mice results in impaired contractility in the GI smooth muscle.[25,26] We identified 34 SRF-regulated proteins in the *Srf* knockout smooth muscle, many of which appear to be contractile and contractile-associated proteins.[10] Furthermore, we found that expression of voltage-activated L-type calcium channel CACNA1C is also regulated by SRF-induced myotonic dystrophy protein kinases (manuscript in revision). In smooth muscle, the excitation-contraction coupling of smooth muscle is mainly regulated by the L-type Ca^{2+} channels.[45] Reduction of CACNA1C in *Srf* knockout SMCs not only decreased intracellular Ca^{2+} spikes, but also disrupted their coupling between cells resulting in decreased contractility.

GI motility is largely regulated by the activities of three electrically coupled cell types, SMCs, interstitial cells of Cajal, and PDGFRα^{+} cells (called SIP), which form a multicellular functional syncytium via gap junctions.[22] Disruption of coordination of these coupled cells alters GI motility patterns.[46,47] Defective SMCs with loss or reduced expression of SRF may impair the SIP activity and GI motility.

Cancer

Although several studies demonstrated that SRF is linked to tumorigenesis, SRF seems to both negatively and positively contribute to GI cancers depending on pathways. The promoter and exon 1 of the *SRF* gene became hypermethylated in gastric carcinoma, which downregulated the mRNA expression.[48] Overall patient survival from gastric carcinoma metastasis in China, Japan, and Korea has been linked to the differential methylation of *SRF*, *GFRA1*, and *ZNF382*.[48] In addition, a truncated SRF isoform, SRFΔ5 appears to be abnormally overexpressed in colon cancer.[20] Over-expression of this isoform increased cell survival, suggesting that this truncated protein may contribute to colon tumorigenesis.[20] However, in the truncated SRF study, it was not made clear whether the truncated protein alone induced cell growth or whether this truncated protein simply attenuated the effect of SRF. The down-regulated SRF that induced gastric cancer[48] suggested the latter may be the case in colon cancer. Another pathway contributing to GI cancers is by the oncogene *FHL2* whose expression is induced by SRF.[8] FHL2 is a cell cycle and growth modulator that is highly expressed in GI cancers such as colon cancer.[49] FHL2 is required for cancer cell invasion, migration, and adhesion to the extracellular matrix.[50] Our SMC genome and CArGome browser[10] identified the SRF binding site and multiple transcriptional variants of *Fhl2* gene (Fig. 1). It is of interest to investigate how this gene is activated by SRF, DNA methylation, or histone modifications in cancer, and which transcriptional variant is responsible for tumorigenesis. Taken together, the positive and negative regulation of genes by SRF in

GI cancers suggests a multifunctional role of SRF in cell phenotype and tumorigenesis.

Ulcer

Other than circular and longitudinal SMCs, SRF is also expressed in the SMCs of the muscularis mucosa, proliferative cells of mucosal epithelium, and endothelial cells of microvessels.[51] SRF is required for the wound healing process in gastric and esophageal ulcers.[21,52] SRF appears to induce VEGF-induced angiogenesis in endothelial cells.[52] Overexpression of SRF in gastric epithelial cells and SMCs promotes proliferation and migration of cells, which lead to re-epithelialization and restoration of smooth muscle structures damaged by ulcers.[21] In addition, SRF is also critical for TGFβ-induced myofibroblast differentiation during esophageal ulcer healing.[53]

Conclusions

SRF is a multifunctional phenotypic modulator that is linked to several GI diseases (Fig. 2). SMCs require a normal expression of SRF for their differentiation. Complete loss of SRF in SMCs induces apoptosis of the cells, which results in degeneration of the muscle. Reduction of SRF in SMCs triggers the cells to dedifferentiate and become proliferative, leading to hypertrophic muscle, gastric and colon cancer. Conversely, overexpression of SRF induces proliferation of cells (endothelial cells, epithelial cells, and SMCs) in ulcer healing that results in re-epithelialization and restoration of smooth muscle.

Future Directions

Given that SRF plays a critical role in controlling SMC behavior in different pathological conditions, the protein is a good therapeutic target gene to aid in recovery from GI injury. For example, normalization of SRF expression may thwart or delay the progression of SMC death in myopathy, SMC dedifferentiation and proliferation in hypertrophy, and cancer cell proliferation and migration in gastric and colon cancers. For ulcers, overexpression of SRF may also accelerate the healing process of re-epithelialization and restoration of smooth muscle. However, we still do not know the identity of SRF susceptible cells that abnormally change their phenotype in ulcers and cancers. Further studies should be performed to identify SRF susceptible cells and target genes in the pathological condi-

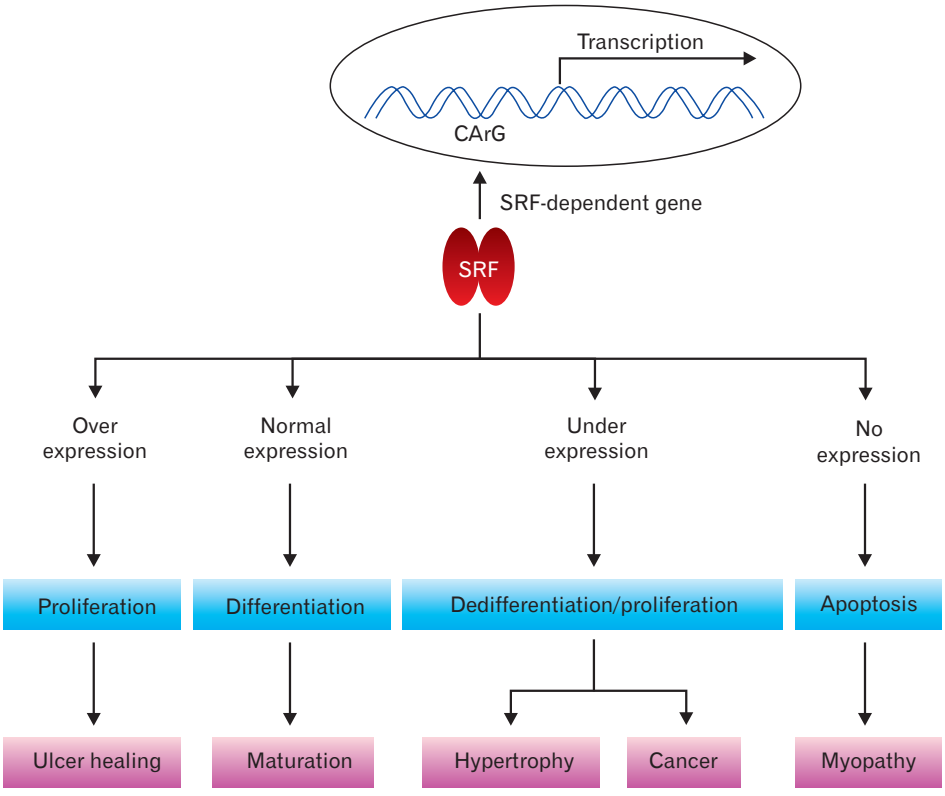

Figure 2. Multi-phenotypic role of serum response factor (SRF) in the digestive system.

tions. In addition, tools for in vivo overexpression or restoration of SRF should be developed. These tools can be vial gene delivery systems, nanoparticles, or chemicals that induce or restore SRF expression. SRF itself induces its own expression. Thus subtle increases in SRF expression may be enough to trigger a positive feedback reaction that would restore SRF in the protein deficient diseases. Demethylation of the hypermethylated SRF gene may provide a new anti-cancer therapy to stop or kill proliferating cancer cells. Although we are still far away from treating SRF deficient diseases,[4] this multi-phenotypic protein could offer potential clinical applications in medicine that can reverse some of the unwanted pathological changes occurring in these GI diseases.

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Conflicts of interest: None.

References

1. Treisman R. Identification of a protein-binding site that mediates transcriptional response of the c-fos gene to serum factors. Cell 1986;46:567-574.
2. Miano JM. Serum response factor: toggling between disparate programs of gene expression. J Mol Cell Cardiol 2003;35:577-593.
3. Owens GK, Kumar MS, Wamhoff BR. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. Physiol Rev 2004;84:767-801.
4. Miano JM. Role of serum response factor in the pathogenesis of disease. Lab Invest 2010;90:1274-1284.
5. Miano JM, Long X, Fujiwara K. Serum response factor: master regulator of the actin cytoskeleton and contractile apparatus. Am J Physiol Cell Physiol 2007;292:C70-C81.
6. Benson CC, Zhou Q, Long X, Miano JM. Identifying functional single nucleotide polymorphisms in the human CArGome. Physiol Genomics 2011;43:1038-1048.
7. Cooper SJ, Trinklein ND, Nguyen L, Myers RM. Serum response factor binding sites differ in three human cell types. Genome Res 2007;17:136-144.
8. Sun Q, Chen G, Streb JW, et al. Defining the mammalian CArGome. Genome Res 2006;16:197-207.
9. Zhang SX, Garcia-Gras E, Wycuff DR, et al. Identification of direct serum-response factor gene targets during Me2SO-induced P19 cardiac cell differentiation. J Biol Chem 2005;280:19115-19126.
10. Lee MY, Park C, Berent RM, et al. Smooth muscle cell genome browser: enabling the identification of novel serum response factor target genes. PLoS One 2015;10:e0133751.
11. Niu Z, Li A, Zhang SX, Schwartz RJ. Serum response factor micromanaging cardiogenesis. Curr Opin Cell Biol 2007;19:618-627.
12. Park C, Lee MY, Slivano OJ, et al. Loss of serum response factor induces microRNA-mediated apoptosis in intestinal smooth muscle cells. Cell Death Dis 2015;6:e2011.
13. Park C, Hennig GW, Sanders KM, et al. Serum response factor-dependent MicroRNAs regulate gastrointestinal smooth muscle cell phenotypes. Gastroenterology 2011;141:164-175.
14. Davis-Dusenbery BN, Wu C, Hata A. Micromanaging vascular smooth muscle cell differentiation and phenotypic modulation. Arterioscler Thromb Vasc Biol 2011;31:2370-2377.
15. Arsenian S, Weinhold B, Oelgeschlager M, Ruther U, Nordheim A. Serum response factor is essential for mesoderm formation during mouse embryogenesis. EMBO J 1998;17:6289-6299.
16. Miano JM, Ramanan N, Georger MA, et al. Restricted inactivation of serum response factor to the cardiovascular system. Proc Natl Acad Sci USA 2004;101:17132-17137.
17. Niu Z, Yu W, Zhang SX, et al. Conditional mutagenesis of the murine serum response factor gene blocks cardiogenesis and the transcription of downstream gene targets. J Biol Chem 2005;280:32531-32538.
18. Park C, Lee M, Park PJ, et al. Serum response factor is essential for prenatal gastrointestinal smooth muscle development and maintenance of differentiated phenotype. J Neurogastroenterol Motil 2015;21:589-602.
19. Parlakian A, Tuil D, Hamard G, et al. Targeted inactivation of serum response factor in the developing heart results in myocardial defects and embryonic lethality. Mol Cell Biol 2004;24:5281-5289.
20. Patten LC, Belaguli NS, Baek MJ, Fagan SP, Awad SS, Berger DH. Serum response factor is alternatively spliced in human colon cancer. J Surg Res 2004;121:92-100.
21. Chai J, Baatar D, Tarnawski A. Serum response factor promotes re-epithelialization and muscular structure restoration during gastric ulcer healing. Gastroenterology 2004;126:1809-1818.
22. Sanders KM, Koh SD, Ro S, Ward SM. Regulation of gastrointestinal motility--insights from smooth muscle biology. Nat Rev Gastroenterol Hepatol 2012;9:633-645.
23. Floyd R, Wray S. Calcium transporters and signalling in smooth muscles. Cell Calcium 2007;42:467-476.
24. Long X, Tharp DL, Georger MA, et al. The smooth muscle cell-restricted KCNMB1 ion channel subunit is a direct transcriptional target of serum response factor and myocardin. J Biol Chem 2009;284:33671-33682.
25. Mericskay M, Blanc J, Tritsch E, et al. Inducible mouse model of chronic intestinal pseudo-obstruction by smooth muscle-specific inactivation of the SRF gene. Gastroenterology 2007;133:1960-1970.
26. Angstenberger M, Wegener JW, Pichler BJ, et al. Severe intestinal obstruction on induced smooth muscle-specific ablation of the transcription factor SRF in adult mice. Gastroenterology 2007;133:1948-1959.
27. Lepore JJ, Cheng L, Min Lu M, Mericko PA, Morrisey EE, Parmacek MS. High-efficiency somatic mutagenesis in smooth muscle cells and cardiac myocytes in SM22alpha-Cre transgenic mice. Genesis 2005;41:179-184.
28. Xin HB, Deng KY, Rishniw M, Ji G, Kotlikoff MI. Smooth muscle expression of Cre recombinase and eGFP in transgenic mice. Physiol Genomics 2002;10:211-215.

29. Miano JM, Cserjesi P, Ligon KL, Periasamy M, Olson EN. Smooth muscle myosin heavy chain exclusively marks the smooth muscle lineage during mouse embryogenesis. Circ Res 1994;75:803-812.
30. Solway J, Seltzer J, Samaha FF, et al. Structure and expression of a smooth muscle cell-specific gene, SM22 alpha. J Biol Chem 1995;270: 13460-13469.
31. Bernstein E, Caudy AA, Hammond SM, Hannon GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 2001;409:363-366.
32. Carmell MA, Hannon GJ. RNase III enzymes and the initiation of gene silencing. Nat Struct Mol Biol 2004;11:214-218.
33. Park C, Yan W, Ward SM, et al. MicroRNAs dynamically remodel gastrointestinal smooth muscle cells. PLoS One 2011;6:e18628.
34. Xin M, Small EM, Sutherland LB, et al. MicroRNAs miR-143 and miR-145 modulate cytoskeletal dynamics and responsiveness of smooth muscle cells to injury. Genes Dev 2009;23:2166-2178.
35. Wiese KE, Haikala HM, von Eyss B, et al. Repression of SRF target genes is critical for Myc-dependent apoptosis of epithelial cells. EMBO J 2015.
36. Sisson TH, Ajayi IO, Subbotina N, et al. Inhibition of myocardin-related transcription factor/serum response factor signaling decreases lung fibrosis and promotes mesenchymal cell apoptosis. Am J Pathol 2015;185:969-986.
37. Bae JS, Noh SJ, Kim KM, et al. Serum response factor induces epithelial to mesenchymal transition with resistance to sorafenib in hepatocellular carcinoma. Int J Oncol 2014;44:129-136.
38. Chen C, Wang Y, Yang S, et al. MiR-320a contributes to atherogenesis by augmenting multiple risk factors and down-regulating SRF. J Cell Mol Med 2015;19:970-985.
39. Huang J, Wang T, Wright AC, et al. Myocardin is required for maintenance of vascular and visceral smooth muscle homeostasis during postnatal development. Proc Natl Acad Sci USA 2015;112:4447-4452.
40. Parmacek MS. Myocardin-related transcription factors: critical coactivators regulating cardiovascular development and adaptation. Circ Res 2007;100:633-644.
41. Wang D, Chang PS, Wang Z, et al. Activation of cardiac gene expression by myocardin, a transcriptional cofactor for serum response factor. Cell 2001;105:851-862.
42. Chen J, Chen H, Sanders KM, Perrino BA. Regulation of SRF/CArG-dependent gene transcription during chronic partial obstruction of murine small intestine. Neurogastroenterol Motil 2008;20:829-842.
43. Wang Z, Wang DZ, Hockemeyer D, McAnally J, Nordheim A, Olson EN. Myocardin and ternary complex factors compete for SRF to control smooth muscle gene expression. Nature 2004;428:185-189.
44. Chevigny M, Guerin-Montpetit K, Vargas A, Lefebvre-Lavoie J, Lavoie JP. Contribution of SRF, Elk-1, and myocardin to airway smooth muscle remodeling in heaves, an asthma-like disease of horses. Am J Physiol Lung Cell Mol Physiol 2015;309:L37-L45.
45. Moosmang S, Schulla V, Welling A, et al. Dominant role of smooth muscle L-type calcium channel Cav1.2 for blood pressure regulation. EMBO J 2003;22:6027-6034.
46. Blair PJ, Rhee PL, Sanders KM, Ward SM. The significance of interstitial cells in neurogastroenterology. J Neurogastroenterol Motil 2014;20:294-317.
47. Sanders KM, Ward SM, Koh SD. Interstitial cells: regulators of smooth muscle function. Physiol Rev 2014;94:859-907.
48. Liu Z, Zhang J, Gao Y, et al. Large-scale characterization of DNA methylation changes in human gastric carcinomas with and without metastasis. Clin Cancer Res 2014;20:4598-4612.
49. Wang J, Yang Y, Xia HH, et al. Suppression of FHL2 expression induces cell differentiation and inhibits gastric and colon carcinogenesis. Gastroenterology 2007;132:1066-1076.
50. Cao CY, Mok SW, Cheng VW, Tsui SK. The FHL2 regulation in the transcriptional circuitry of human cancers. Gene 2015;572:1-7.
51. Chai J, Baatar D, Moon W, Tarnawski A. Expression of serum response factor in normal rat gastric mucosa. J Physiol Pharmacol 2002;53:289-294.
52. Chai J, Jones MK, Tarnawski AS. Serum response factor is a critical requirement for VEGF signaling in endothelial cells and VEGF-induced angiogenesis. FASEB J 2004;18:1264-1266.
53. Chai J, Norng M, Tarnawski AS, Chow J. A critical role of serum response factor in myofibroblast differentiation during experimental oesophageal ulcer healing in rats. Gut 2007;56:621-630.