Role of NRSF/REST in the Regulation of Cardiac Gene Expression and Function
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Alterations in the cardiac gene program affect both cardiac structure and function, and play a key role in the progression of pathological cardiac remodeling and heart failure. For instance, reactivation of fetal cardiac genes in adults is a consistent feature of cardiac hypertrophy and heart failure. Investigation of the transcriptional regulation of cardiac genes revealed a transcriptional repressor, neuron-restrictive silencer factor (NRSF), also called repressor element-1 silencing factor (REST), to be an important regulator of multiple fetal cardiac genes. Inhibition of NRSF in the heart leads to cardiac dysfunction and sudden arrhythmic death accompanied by re-expression of various fetal genes, including those encoding fetal ion channels, such as the HCN channels and T-type Ca\(^{2+}\) channels. These findings shed light on the crucial regulatory function of NRSF in the heart and its importance for maintaining normal cardiac integrity. (Circ J 2013; 77: 2682–2686)

Key Words: Atrial natriuretic factor; Brain natriuretic peptide; Heart failure; Remodeling

Cardiac hypertrophy and heart failure are leading causes of morbidity and mortality worldwide.\(^1\) Cardiac myocytes hypertrophy in response to pathological stress on the heart, which can be in the form of mechanical stress, tissue injury or neurohumoral stimulation.\(^2\) Initially, hypertrophy likely functions as an adaptive response, but when the stress is prolonged, the heart undergoes pathological remodeling and hypertrophy, ultimately leading to heart failure.

Cardiac hypertrophy is accompanied by increases in cell size, protein synthesis and sarcomeric assembly, and alterations in gene expression. Among these, the reactivation of fetal cardiac genes, such as those encoding atrial and brain natriuretic peptides (ANP and BNP, respectively), fetal isoforms of contractile proteins (skeletal α-actin and β-myosin heavy chain), fetal-type cardiac ion channels and some smooth muscle proteins (smooth muscle α-actin and smooth muscle 22α), is a consistent marker of cardiac hypertrophy and pathological remodeling.\(^3,4\) These genes are abundantly expressed in fetal ventricles, but become quiescent after birth as the heart matures. Furthermore, reactivation of the fetal gene program is not merely a marker of pathological conditions; by altering cardiac structure and function, it also plays an important role in the molecular process underlying pathological cardiac remodeling.\(^5,6\)

Numerous transcriptional factors are involved in the regulation of the fetal cardiac gene program,\(^6\) and of these, I am focusing on the role of the transcriptional repressor neuron-restrictive silencer factor (NRSF), also called repressor element-1 silencing transcription factor (REST). NRSF has been shown to negatively regulate the gene transcription of ANP and BNP,\(^7,8\) as well as several other fetal cardiac genes, including CACNA1H and HCN2 and -4, which encode the T-type Ca\(^{2+}\) channel and 2 hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, respectively. This suggests NRSF participates in the molecular pathways regulating the fetal cardiac gene program.\(^9\) Consistent with that idea, cardiac-restricted inactivation of NRSF through overexpression of a dominant-negative NRSF mutant (dnNRSF) driven by the cardiac-specific α-MHC promoter in transgenic mice (dnNRSF-Tg mice) leads to upregulation of fetal cardiac gene expression in the ventricle. What’s more, dnNRSF-Tg mice exhibit cardiomyopathy and sudden arrhythmic death, demonstrating that by negatively regulating cardiac gene expression, NRSF contributes to the maintenance of normal cardiac integrity.\(^9\) Here I review the role played by NRSF in the regulation of cardiac gene expression and the maintenance of the normal cardiac phenotype.

NRSF as Transcriptional Repressor of ANP and BNP Gene Expression

Studies of transgenic mice carrying a reporter gene fused to an approximately 2.4-kbp fragment of the 5' flanking region (5'-FR) of the human ANP gene, or an approximately 3-kbp fragment of the 5'-FR of the rat ANP gene, show that the proximal 5'-FR of ANP is sufficient to spatially and temporally recapitulate endogenous ANP expression. This reflects that fact that 5'-FR of ANP contains conserved elements for several transcriptional factors important in the regulation of ANP expression. However, there are some differences in the expression pattern between the proximal 5'-FR of ANP and the endogenous ANP, suggesting important regulatory elements also lie outside the proximal 5'-FR.\(^10\) For example, a fragment containing the 3'-untranslated region (UTR) of human ANP...
This suggests that loss of NRSE-dependent repression is at least partially responsible for the hypertrophic stimulus-inducible expression of ANP in cardiac myocytes.

Analysis of the human BNP promoter has revealed the presence of a sequence similar to the NRSE in the 5'-UTR of ANP. This sequence, called the fibronectin-inducible element, is well conserved among species and mediates fibronectin-induced increases in BNP promoter activity in cardiac myocytes (Figure 1). In addition, gel shift assays have confirmed that it binds NRSF, and mutation of the sequence significantly increases human BNP promoter activity, indicating that NRSF suppresses expression of BNP as well as ANP.

Similarly, adenovirus-mediated expression of dnNRSF leads to increased expression of both ANP and BNP in cultured ventricular myocytes, and ventricular expression of both ANP and BNP is upregulated in dnNRSF-Tg mice. Adenovirus-mediated dnNRSF expression also attenuates the induction of ANP and BNP expression of the gene's 5'-UTR. Within the 3'-UTR of ANP, there is a conserved sequence very similar to the consensus sequence of the neuron-restrictive silencer element (NRSE), to which the transcriptional repressor NRSF binds (Figure 1).

NRSF was originally identified as a transcriptional regulator that suppresses the expression of neuron-specific genes in non-neuronal cells and is widely expressed in non-neuronal tissues, including the adult heart. Later it became evident that in addition to neuron-specific genes, NRSF also suppresses non-neuronal genes that contain NRSE, indicating that NRSF functions as a transcriptional regulator of both neuronal and non-neuronal genes containing NRSE in non-neuronal tissues. In ANP, mutation of the NRSE in the 3'-UTR results in complete loss of repressor activity toward human ANP. NRSE mutation also diminishes the increase in ANP promoter activity normally stimulated by endothelin-1 in cultured ventricular myocytes. This suggests that loss of NRSE-dependent repression is at least partially responsible for the hypertrophic stimulus-inducible expression of ANP in cardiac myocytes.

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Figure 3. Nuclear export of class IIa HDACs following activation of pathological signaling mediates relief of NRSF-mediated gene repression during cardiac remodeling. HDAC, histone deacetylase; NRSE, neuron-restrictive silencer element; NRSF, neuron-restrictive silencer factor; REST, repressor element-1 silencing factor.

expression in response to hypertrophic stimuli. For example, the increases in ANP and BNP expression normally elicited by pressure overload are significantly diminished in hearts expressing dnNRSF. Conversely, forced recruitment of NRSF to the ANP promoter in cardiac myocytes reduces basal promoter activity, but enhanced the response to endothelin-1. Thus relief of NRSF-mediated repression appears to contribute to the pathological stimulus-inducible expression of ANP and BNP.

NRSF Regulation of Cardiac Gene Expression Through Association With Epigenetic Machinery

Numerous studies using non-cardiac cells have shown that NRSF mediates transcriptional repression by recruiting corepressor complexes. NRSF contains 2 repressor domains respectively located in its N- and C-terminal regions. The N-terminal repressor domain is known to interact with the mSin3A and mSin3B complexes, which associate with histone deacetylases (HDACs), while the C-terminal repressor domain interacts with the corepressor CoREST, which forms a complex with HDACs, the histone demethylase LSD1, and the ATP-dependent chromatin remodeling enzyme BRG1 (Figure 2). HDACs are transcriptional corepressors that promote nucleosomal condensation and consequently repress transcription. LSD1 demethylates mono- and dimethylated lysines, specifically histone 3, lysine 4 and 9, and represses gene expression. Within cardiac myocytes, NRSF complexes with class I HDACs and with the class IIa HDACs HDAC4 and 5, thereby repressing ANP and BNP expressions through modification of histone acetylation. Whereas class I HDACs are expressed relatively ubiquitously, expression of class IIa HDACs, which include HDAC4, -5, -7 and -9, is tissue-specific, with the highest expression in heart, brain and skeletal muscle, where they reportedly act as signal-responsive repressors of cardiac hypertrophy. In addition to modification of histone acetylation, regulation of histone H3 lysine methylation is also involved in the NRSF-mediated repression of ANP and BNP expression.

On the other hand, hypertrophic stimulus-induced phosphorylation by Ca2+/calmodulin-dependent kinase and/or protein kinase D causes nuclear export of HDACs, which likely contributes to the relief of NRSF-mediated repression of the fetal gene program during cardiac remodeling (Figure 3). Taken together, these findings indicate that NRSF regulates cardiac gene expression through an association with the epigenetic machinery (Figure 2). Further studies of the precise mechanisms by which each component of the epigenetic machinery contributes to NRSF-mediated control of the cardiac gene program will further our understanding of the molecular mechanisms underlying the altered gene expression seen during pathological cardiac remodeling.

NRSF and Maintenance of Normal Cardiac Structure and Function

The gene encoding skeletal α-actin also contains NRSE in its 3′-UTR. Skeletal α-actin gene is expressed in the fetal ventricle, but its ventricular expression declines after birth and is only re-induced in response to pathological stress. This implies that NRSF regulates the expression of multiple fetal cardiac genes, thereby contributing to the maintenance of cardiac structure and function. As would be expected, the expression of the skeletal α-actin gene, as with ANP and BNP, is significantly upregulated in dnNRSF-Tg mice. More interestingly, dnNRSF-Tg mice show early mortality because of depressed cardiac systolic function, left ventricular dilation and sudden arrhythmic death. NRSF thus appears essential for the maintenance of the normal cardiac phenotype, most likely through regulation of the cardiac gene program. Furthermore, deletion of guanylyl cyclase-A, a common receptor for ANP and BNP, in dnNRSF-Tg exacerbates pathological remodeling, demonstrating that it is not the increased expression of ANP and BNP that is responsible for the cardiomyopathy and sudden arrhythmic death seen in dnNRSF-Tg mice. Considering the involvement of NRSF in the regulation of the fetal cardiac gene program, further elucidation of the mechanisms underlying the cardiac dysfunction and electrical instability in dnNRSF-Tg mice may lead to identification of the molecular mechanisms involved in pathological cardiac remodeling.

NRSF and Regulation of HCN and T-type Ca2+ Channels

The observation that dnNRSF-Tg mice experience malignant arrhythmias and sudden arrhythmic death likely reflects elec-
trical instability related altered ion channel activity. To date, 2 types of fetal cardiac ion channel, HCN and T-type Ca\(^{2+}\) channels, have been identified as potentially involved in the increased arrhythmia seen in dnNRSF-Tg mice.

**HCN Channels**

The HCN ion channel family (HCN1–4) carries the I\(_f\) current.\(^{20,21}\) In the adult heart, HCN channels are predominantly expressed in the conduction system, especially the sinoatrial node, where HCN4 is the major isoform and controls cardiac rhythmicity.\(^{21}\) HCN channels are also expressed in ventricular myocytes, where HCN2 is the dominant isoform, but the level of HCN channel expression in the adult ventricular myocardium is normally much lower than in the conduction system. In fact, I\(_f\) is barely detectable in normal ventricular myocytes.\(^{21}\) During development, HCN channels are abundantly expressed in the fetal ventricular myocardium, but their ventricular expression progressively declines after birth until it is largely restricted to the conduction system in the adult heart.\(^{22}\) Expression levels of both HCN2 and -4 genes are significantly elevated in dnNRSF-Tg hearts, and conserved NRSE-like sequences have been identified in the first intron of both genes.\(^{9}\) When expressed in ventricular myocytes, a fragment of the first intron of HCN4 containing NRSE significantly repressed HCN4 promoter activity in a NRSE-dependent manner.\(^{23}\) Furthermore, the expression profile of NRSE mRNA is anti-parallel to that of HCN4 during cardiac development, suggesting NRSE regulates the developmental changes of HCN4 expression. In contrast to NRSE, the transcriptional activator MEF-2, whose binding sequence is also located within the first intron of HCN4, activates HCN4 promoter activity in cardiac myocytes.\(^{24}\) HCN2 and -4 genes are re-expressed in hypertrophied and failing ventricles in both rodents and humans.\(^{21,25}\) and NRSE appears to play an important role in mediating the re-induction of HCN4 expression in response to hypertrophic stimuli.\(^{9,23}\) These results thus suggest that NRSE regulates both the basal and inducible expression of the HCN2 and -4 genes in ventricular myocytes (Figure 4).

To further explore the contribution made by HCNs to the pathophysiology of dnNRSF-Tg mice, the animals were treated with ivabradine, a specific HCN blocker.\(^{26}\) The heart rates of dnNRSF-Tg mice are slower than those of wild-type mice. Moreover, at a dose of 7 mg·kg\(^{-1}\)·day\(^{-1}\), ivabradine significantly suppressed ventricular tachycardias and prolonged survival without reducing heart rate or affecting cardiac structure or systolic function. In ventricular myocytes from dnNRSF-Tg mice, ivabradine acts by reducing the abnormally increased automaticity, thereby suppressing the trigger for arrhythmias. Conversely, cardiac-specific overexpression of HCN2 increased the number of ventricular tachycardias, despite treatment with isoproterenol, a \(\beta\)-receptor antagonist. These results suggest that increased ventricular HCN expression is responsible for the enhanced arrhythmia seen in dnNRSF-Tg mice (Figure 4). It is noteworthy that increased expression of the HCN2 and -4 genes has also been observed in hypertrophied and failing human ventricles. The SHIFT trial found that the cardioprotective effects exerted by ivabradine are associated with a reduction in heart rate;\(^{27,28}\) however, it also appears that ivabradine can have a cardioprotective effect independent of heart rate reduction.

**T-type Ca\(^{2+}\) Channels**

Another well-studied fetal cardiac ion channel is the T-type Ca\(^{2+}\) channel.\(^{29}\) One of the major sources of Ca\(^{2+}\) influx in excitable cells is voltage-gated Ca\(^{2+}\) channels, which have been classified into several types: L-(long lasting), T-(transient), N-(neuronal), P/Q-(Purkinje) and R-(residual-drug resistant); generally, cardiac myocytes express only the L- and T-types. L-type Ca\(^{2+}\) channels predominate in mature cardiac myocytes and are crucially involved in excitation-contraction coupling.\(^{30}\) During development, T-type Ca\(^{2+}\) channels are abundantly expressed in the embryonic ventricle, but as with HCN channels, their expression is repressed in the adult ventricle such that they are restricted to the conduction system.\(^{31,32}\) and are only re-expressed in hypertrophied and failing hearts.\(^{33}\) The CACNA1H gene, which encodes the \(\alpha\)1-subunit of T-type Ca\(^{2+}\) channels, contains an NRSE-like sequence in its first intron. This sequence has 93% identity with the consensus NRSE sequence and is well conserved among species, including humans. NRSE binds to the sequence, suggesting NRSE negatively regulates CACNA1H expression in cardiac ventricular myocytes.\(^{3}26\) Consistent with that idea, CACNA1H expression is increased in dnNRSF-Tg ventricles, and an increase in T-type Ca\(^{2+}\) currents has been detected in ventricular myocytes isolated from dnNRSF-Tg hearts (Figure 4). This suggests inhibition of NRSE-mediated repression contributes to the increased cardiac expres-

**Figure 4.** NRSE contributes to the regulation of the cardiac gene program by collaborating with other transcription factors, thereby maintaining normal cardiac systolic function and electrical stability. Re-activation of fetal-type cardiac ion channels, such as the HCN and T-type Ca\(^{2+}\) channels, contributes to the electrical instability observed in mice in which NRSE function is disrupted (dnNRSF-Tg mice). HCN, hyperpolarization-activated cyclic nucleotide; NFAT, nuclear factor of T cells; MEF2, myocytes enhancer factor 2; NRSE, neuron-restrictive silencer factor.
Contribution of T-type Ca\(^{2+}\) channels seen under pathological conditions. Pharmacological inhibition of T-type Ca\(^{2+}\) currents significantly improves survival among dnNRSF-Tg mice while reducing electrical abnormalities in ventricular myocytes, and thus the incidence of arrhythmias in dnNRSF-Tg hearts. This suggests T-type Ca\(^{2+}\) channels could be a useful therapeutic target for the treatment of heart failure with malignant arrhythmias.\(^{34}\)

**Conclusion**

Genetic reprogramming contributes to progression of heart failure through effects on myocardial cellular function and structure. The transcriptional repressor NRSF inhibits the expression of multiple fetal cardiac genes through recruitment of HDACs and other epigenetic factors. Inhibition of NRSF in the heart leads to left ventricular enlargement, cardiac dysfunction and sudden arrhythmic death accompanied by re-expression of multiple fetal cardiac genes, including those encoding fetal ion channels (HCN and T-type Ca\(^{2+}\) channels). Conversely, inhibiting fetal ion channels significantly prolongs survival in dn-NRSF-Tg mice by reducing the incidence of malignant arrhythmias. These results indicate that NRSE regulates the cardiac gene program, thereby maintaining normal cardiac integrity (Figure 4). It is anticipated that further study of the molecular mechanisms by which NRSF regulates the cardiac gene program and participates in the development of pathological cardiac remodeling will improve our understanding of disease processes in the heart and shed light on potential therapeutic targets for the treatment of heart failure.

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**Disclosures**

Conflict of interest: the author has no conflicts of interest to declare.

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