

Regulation of SCN5A by Non-coding RNAs in the Brugada Syndrome Context

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Abstract

In the human heart, the action potential (AP) is initiated and maintained thanks to a fast-activating fast-inactivating Na⁺ current carried by Nav1.5 channels. The pivotal physiological role of Nav1.5 in the heart is reflected by the important consequences of its coding SCN5A gene mutations. These mutations may lead to an impaired functional expression (including expression level, subcellular localization, trafficking, and/or current density), and are generally correlated with severe cardiac rhythm disorders such as Long QT (LQT) and Brugada syndrome (BrS). In BrS, loss of function mutations in SCN5A account for 35-40% of clinically affected patients and around 400 mutations in the SCN5A gene were identified in probands with BrS. Emerging electrophysiological techniques such as patch clamp along with transgenic animal technologies improved our understanding of the pathogenic mechanisms underlying BrS due to SCN5A variants. However, despite significant advances in defining the pathophysiology of Nav1.5, the molecular mechanisms underlying its regulation and contribution to the disease are poorly understood. It is well established that functional expression of Nav1.5 may be under modulation by post-transcriptional regulators, defining thus its transcript levels in the cell and also the penetrance on its associated diseases. Recently, non-coding RNA (ncRNAs) molecules have been identified as key transcriptional regulators of SCN5A expression in the heart. The present mini-review provides a summary of the role of ncRNAs, especially microRNAs (miRNAs), in the regulation of SCN5A. It mainly focuses on their role in the BrS context and discusses the recent updates and the major gaps still to be elucidated.

Abbreviations

AP: Action potential; LQT: Long QT; BrS: Brugada syndrome; ncRNAs: non-coding RNA; miRNAs: microRNAs; lncRNAs: long non coding RNAs; 3'UTR: 3' untranslated region; SNPs: Single-nucleotide polymorphisms.

Introduction

The tetrodotoxin (TTX) resistant isoform Nav1.5, encoded by the *SCN5A* gene, is the predominant isoform of voltage-gated sodium (Na⁺) channels in the heart¹. This channel plays a key role generating the cardiac action potential and maintaining the rapid conduction of electrical signals through cardiac tissues². Its involvement in the aetiology of numerous cardiac physiopathologies strongly suggests that proper regulation of cell biology and function of the channel is critical for normal cardiac function³. The physiological importance of Nav1.5 in the heart is further reflected by the important consequences of variants (mutations) in its coding gene *SCN5A*⁴. Regardless their position in the gene sequence, these mutations may lead to an impaired functional expression (including expression

level, subcellular localization, trafficking, and/or current density), and are generally correlated with severe cardiac rhythm disorders such as Long QT (LQT) and Brugada syndrome (BrS)^{5,6}.

Long QT syndrome is a primary electrical disease characterized by prolongation of the repolarization process reflected on the electrocardiogram (ECG) as a prolongation of the QT interval. The disease is associated with potentially lethal ventricular arrhythmias. Genetically, LQT is classified into 17 different genetic subtypes including the SCN5A related type (LQT3) which represents up to 10% of LQT reported cases. Nav1.5 mutations in LQT3 display a gain-of-function either by a pathological increase in I_{NaL} or window current or both⁷.

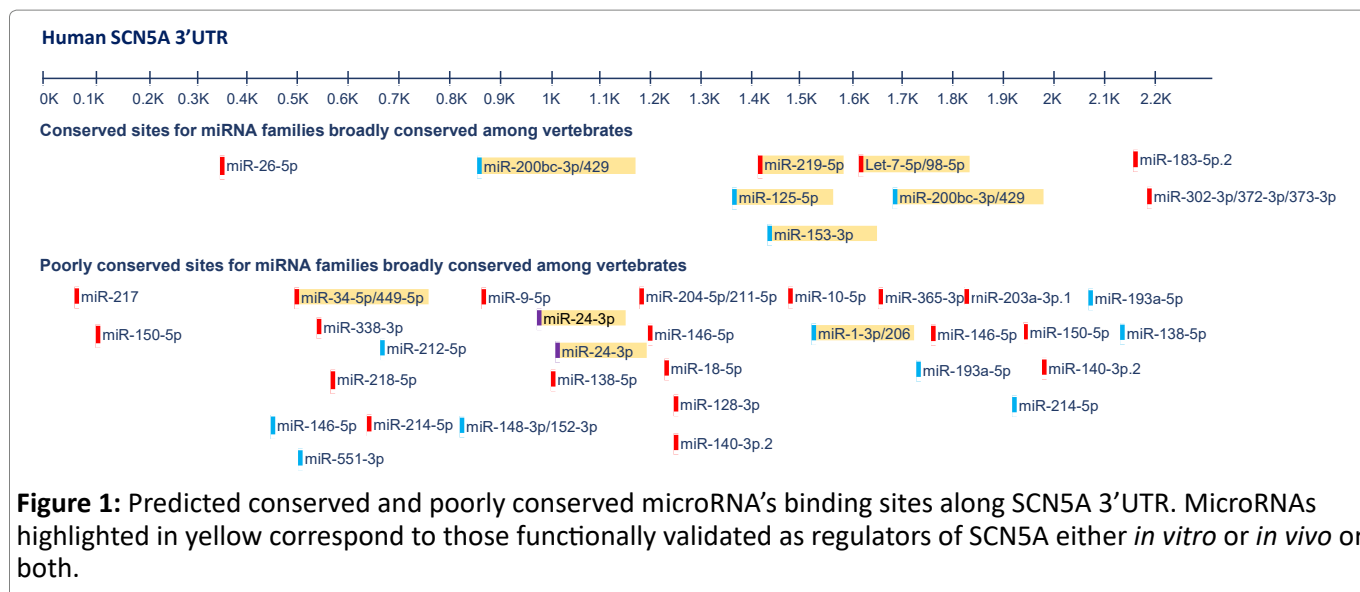
Brugada syndrome is an arrhythmic disorder characterized by a coved type ST-elevation and T wave inversion in the right precordial lead V1 and/or V2 of the ECG consistent with the type1 Brugada ECG pattern and is associated with an increased risk for ventricular fibrillation and sudden cardiac death⁸. BrS is inherited on an autosomal dominant pattern and pathogenic variants in SCN5A are beyond 40% of the pathophysiological substrate of BrS in the affected patients⁹. In some of the cases who tested negative for SCN5A disease-causing variants using conventional sequencing methods, large-scale genomic imbalances (Copy Number Variants, CNVs) have been identified¹⁰. SCN5A mutations in the BrS context are most likely associated with Nav1.5 loss-of-function either by decreased expression of Nav1.5 in the sarcolemma, expression of non-functional channels or altered gating properties leading to a decreased I_{Na} (e.g., delayed activation or earlier or faster inactivation)¹¹. With the recent advances in molecular biology, it became well established that functional expression of Nav1.5 is also under modulation of post-transcriptional regulators, defining its cellular transcript levels and the penetrance of its associated diseases, particularly BrS¹². However, given the complex nature of this sizable gene (nearly 30 exons spanning > 80 kb), SCN5A transcript regulatory mechanisms are far from being totally deciphered although the relentless efforts in the field. An important mechanism affecting the functional expression of SCN5A could be the alternative splicing of wild type and/or mutant Nav1.5 transcripts. Alternative splicing of Nav1.5 creates a total of nine transcripts (Nav1.5a, Nav1.5b, Nav1.5c, Nav1.5d, Nav1.5e, Nav1.5f, truncated variants E28B-D) differentially expressed in the mammalian myocardium¹ and in various other tissues including brain, neuronal cell lines^{13,14}, dorsal root ganglia (DRG)^{15,16}, gastrointestinal tract¹⁷ and various tumor tissues and cell lines^{18,19}. In the heart, functional consequences of Nav1.5 splicing are diverse. Spliced channels can show altered kinetics (Nav1.5a, Nav1.5d, Nav1.5e), they can be non-functional (Nav1.5b, Nav1.5f,

truncated variants E28B-D), or their electrophysiological properties can be virtually unchanged (Nav1.5a, Nav1.5c), when compared to non-spliced Nav1.5¹. Although alternative splicing is demonstrated to be a potential mechanism that allows generating a variety of functionally distinct Nav1.5 channels, yet scarce information is known on the regulation of the expression and function of all five functional Nav1.5 variants in different heart regions, at distinct developmental stages, and in the diseased heart. In addition, the regulatory mechanisms of SCN5A/Nav1.5 seem to be even more complex with the discovery of non-coding RNAs (ncRNAs) and their wide implication in multiple disease states, including cardiac arrhythmogenic pathologies. Recently, ncRNAs molecules have been introduced as key regulators of SCN5A expression in the heart²⁰.

Non-coding RNAs are commonly considered as RNA transcripts that, although not having a direct protein-coding potential, are biologically functional RNAs with fine-tuned mechanisms of protein-coding gene regulation²¹. Considering the complexity of such ncRNAs in structures, genomic orientation, function, cellular localization, or other emerging criteria, a simple and common ncRNAs classification was raised²². The most well-accepted classification model was based on the length of RNA: small non coding RNAs (shorter than 200 nucleotides in length) and long non coding RNAs (lncRNAs) (longer than 200 nucleotides in length). Small non-coding RNAs contain distinct subclasses such as piwi-RNAs, snoRNAs, siRNAs, and the most numerous and well-studied group of microRNAs^{23,24}.

MicroRNAs are small non-coding RNAs of 22–24 nucleotides that are capable of regulating gene expression via translational repression and mRNA degradation predominantly by binding to the 3' untranslated region (3'UTR) of specific mRNAs^{25,26}. lncRNAs consist of at least 200 nucleotides with limited or no protein-coding capacity²⁷. Due to their ability to interact with DNA, RNA, and proteins, lncRNAs are able to regulate very diverse cellular processes such as chromatin modification, transcription, post-transcriptional modifications, scaffolding, and post-transcriptional mRNA regulation^{28,29}. This way, lncRNAs regulate a wide range of biological processes through their crosstalk with miRNAs that, in turn, regulate mRNAs³⁰. Since these cross talking molecules are so closely related, abnormal expression of one of them interferes with mRNA expression patterns and vice versa, an alteration of mRNA expression, most likely due to genetic variants (mutation), create a dysregulation of the interfering ncRNAs that can culminate in disease development as it is the case of several cardiac disorders³¹.

In the present mini-review we will explain the role of ncRNAs, especially microRNAs (miRNAs), in the regulation



of SCN5A. We will focus on their role in the BrS context and discuss the recent updates and the major gaps still to be elucidated.

Regulation of SCN5A by ncRNAs

In the whole genome network analysis, SCN5A was one of the targets identified to be highly regulated by ncRNAs, however, microRNAs are so far the only ncRNA category that have been described in association with SCN5A³². In this regard, our group has performed a screening of the microRNAs predicted to target SCN5A (**Figure 1**). Using HL-1 cardiomyocytes and luciferase assays, we demonstrated that miR-98, miR-106, miR-200 decrease, and miR-219 increase SCN5A expression levels through directly targeting its 3'UTR, while miR-125 and miR-153 indirectly modulate SCN5A expression through an unknown mechanism. Out of these miRNAs, we demonstrated that miR-219 exceptionally upregulate SCN5A causing an increase in the sodium current *in vitro* and *in vivo* miR-219 administration was capable of correcting the QRS prolongation induced by flecainide intoxication in mice²⁰. Furthermore, we demonstrated that miR-219 and miR-200 display a complementary regulatory pattern on SCN5A, in line with their developmental expression profiles.

A similar study performed by Poon et al. further focused on miR-200c and showed that knockdown or overexpression of this microRNA is associated with changes in SCN5A levels³³. Mazzone and co-workers identified four microRNAs with a possible effect on SCN5A. These microRNAs are miR-200a-3p and miR-429 belonging to the miR-200 cluster and Let7-e and Let7-f belonging to the miR-98 cluster. Particularly, let-7f overexpression was shown to decrease SCN5A expression, Nav1.5 current density and electrical excitability in human smooth muscle cells³⁴. Let-7f along with several other microRNAs such as

miR-378, miR-125a/b, miR-192-5p, miR-34b/c and miR-1270 are proven to target SCN5A by a direct binding to its 3'UTR and upregulation of these miRNAs is anticipated to cause reduction of I_{Na} via downregulating SCN5A/Nav1.5 in the cardiac cells³⁵⁻³⁸.

Lastly, Yang et al. revealed that miR-1 is able to repress SCN5A, distinctly to our previous data showing that miR-1 over-expression in HL-1 atrial cardiomyocytes resulted in a decrease of GJA1 and KCNJ2 levels but not SCN5A^{39,40}. Thus, additional experiments will be required to solve this controversy.

Role of SCN5A-microRNA crosstalk in Brugada syndrome

Causative variants in SCN5A account for 35–40% of individuals with a clinical diagnosis of BrS⁴¹. These variants can be divided into two categories, based on gene location of the variant: SCN5A coding sequence variants, which include rare or common exonic variants and noncoding sequence variants, which include intronic and 3'/5' UTR noncoding variants⁴². To date, only rare coding variants in the SCN5A gene have been significantly associated with the syndrome. However, the genotype/phenotype studies conducted in families with SCN5A mutations illustrate the complex mode of inheritance of BrS. This genetic complexity has recently been confirmed by the identification of common polymorphic alleles strongly associated with disease risk⁴³. Even though, noncoding sequence variants remain the major single-nucleotide polymorphisms (SNPs) identified in the disease context, including also BrS⁴⁴. We previously demonstrated that among the SCN5A variants we have identified in BrS patients, 70% are noncoding genomic variants mainly localized in the 3'UTR^{45,38}.

However, although noncoding sequence variants shape the majority of the BrS genetic background, characterizing

their functional effect in the BrS onset remains the major challenge; SCN5A genetic screening and functional characterization either through computational simulation or functional *in vitro/in vivo* testing generally prioritizes variants in the protein-coding portions and flanking intronic regions of the gene⁴⁵.

Few emerging studies, including ours, have called the attention to the fact that non-coding sequence variants may modulate the clinical phenotype in BrS by affecting the Nav 1.5 expression level as well as its biophysical properties^{38,41}. Through screening several families affected by BrS, we were able to demonstrate that noncoding SCN5A variants could modulate the expression levels of SCN5A in the affected patients by altering the affinity (decreasing or increasing) of regulatory microRNAs to the 3'UTR of the gene³⁸. In this regard, we have identified through bioinformatics analyses three predicted conserved binding sites for miR-1270 within the SCN5A 3'UTR and demonstrated that the presence of rs4073797 and rs4073796 polymorphisms in the SCN5A 3'UTR may create a fourth binding site for miR-1270 in a genetically conserved region. Our functional assays in HL1 cardiomyocytes demonstrated that miR-1270 overexpression decreases Scn5a expression hypothesizing thus that genetic variants creating a new binding site for miR-1270 in the 3'UTR further decrease SCN5A expression and thus contribute to the genetic bases of BrS.

For long time, gene noncoding sequences were considered as the exclusive binding regions of microRNAs at the target's transcripts. Coding sequences were often neglected in microRNA-related studies. Recently, Zhang et al. revealed that the terminal coding exon of SCN5A is functionally engaged by miR-24 which exerts a negative control on cardiac SCN5A expression⁴⁶. Although miR-24 in this study was not investigated in the BrS context, the SCN5A-miR-24 interaction was proved to be altered by the common SCN5A polymorphism D1819D (rs1805126), a polymorphism widely studied in arrhythmic patients particularly those with BrS^{47,48}.

Putative role of lncRNAs in BrS: candidate genes as liaison

As previously said, lncRNAs are able to regulate very diverse cellular processes such as chromatin modification, transcription, post-transcriptional modifications, scaffolding, and post-transcriptional mRNA regulation. Of particular importance is the fact that lncRNAs can modulate the expression and function of microRNAs by acting as endogenous competitive molecules. To date, no lncRNA has been reported to directly or indirectly modulate SCN5A/Nav1.5 expression and/or function. Nonetheless it is important to highlight in this context that other BrS associated genes such as MYH7, IRX5 and CACNA1C have been reported to be regulated by lncRNAs. lncRNA *Myheart*

has been recently reported to be significantly elevated in the blood from AMI patients compared to healthy controls and to regulate MYH6/MYH7 expression by interacting with chromatin remodelling factors in pathological hypertrophy rat models⁴⁹. *CRNDE* lncRNA modulates IRX5 expression in distinct human oncogenic processes⁵⁰⁻⁵³ by different mechanisms, including regulation of miR-136-5p in hepatocellular carcinoma⁵⁴. Most importantly *KCNQ10T1* lncRNA modulates miR-384b/CACNA1C expression in an angiotensin II-induced mouse model of atrial fibrillation⁵⁵ and *TCONS_0075467* regulates miR-328/CACNA1C expression in a rabbit atrial fibrillation model⁵⁶. While no evidence of SCN5A regulation in the BrS context by lncRNAs have yet emerged, these recent reports of the functional role of lncRNAs on BrS associated genes, of particularly importance those of CACNA1C in arrhythmogenic disorders, warrants that their roles in BrS will soon be elucidated.

Conclusions

BrS is characterized by coved-type ST-segment elevation in the right precordial leads (V1-V3)⁸. Although associated with a typical ECG, the diagnosis of BrS is still challenging. First, due to its complex clinical spectrum specially in presence of asymptomatic patients. At the molecular level, BrS is even more complex. Many candidate genes have been identified so far but still SCN5A is considered the major one (35-40% of the BrS cases)⁹. Furthermore, the penetrance of BrS is incomplete which make it difficult to establish the genotype phenotype correlation. The incomplete penetrance has been recently attributed to possible transcriptional regulatory mechanisms making a damaging mutation in one BrS patient, tolerated in the others¹².

Given the complex nature of SCN5A, it is no surprise to have multilayer transcriptional and post-transcriptional regulatory mechanisms governing its expression. With the discovery of ncRNAs and their wide implication in multiple disease states, including cardiac arrhythmias, more evidences are added to their possible role as key regulators of SCN5A expression in the BrS. Among all ncRNA subclasses, microRNAs are so far the only ones being described as post-transcriptional regulators of SCN5A/BrS either downregulating (eg. miR-200) or upregulating (eg. miR-219) its expression^{20,32}. MicroRNAs target SCN5A by binding to its 3'UTR or even by binding to its coding sequence as in the case of miR-24⁴⁶. To date, no evidence of SCN5A regulation in the BrS context by lncRNAs have yet emerged however, the reported functional role of lncRNAs on BrS associated genes suggests that the implication of these ncRNAs in BrS is highly possible. In light of these increasingly emerging instances highlighting how ncRNA dysregulation is tightly linked to the pathogenesis of many human cardiac disorders, including BrS, the potential use

of ncRNAs as promoting therapeutic targets and diagnostic tools became more and more interesting.

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