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Transcriptional regulation of the sodium channel gene (*SCN5A*) by GATA4 in human heart

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ABSTRACT

Aberrant expression of the sodium channel gene (*SCN5A*) has been proposed to disrupt cardiac action potential and cause human cardiac arrhythmias, but the mechanisms of *SCN5A* gene regulation and dysregulation still remain largely unexplored. To gain insight into the transcriptional regulatory networks of *SCN5A*, we surveyed the promoter and first intronic regions of the *SCN5A* gene, predicting the presence of several binding sites for GATA transcription factors (TFs). Consistent with this prediction, chromatin immunoprecipitation (ChIP) and sequential ChIP (*Re*-ChIP) assays show co-occupancy of cardiac GATA TFs GATA4 and GATA5 on promoter and intron 1 *SCN5A* regions in fresh-frozen human left ventricle samples. Gene reporter experiments show GATA4 and GATA5 synergism in the activation of the *SCN5A* promoter, and its dependence on predicted GATA binding sites. *GATA4* and *GATA6* mRNAs are robustly expressed in fresh-frozen human left ventricle samples as measured by highly sensitive droplet digital PCR (ddPCR). *GATA5* mRNA is marginally but still clearly detected in the same samples. Importantly, *GATA4* mRNA levels are strongly and positively correlated with *SCN5A* transcript levels in the human heart. Together, our findings uncover a novel mechanism of GATA TFs in the regulation of the *SCN5A* gene in human heart tissue. Our studies suggest that GATA5 but especially GATA4 are main contributors to *SCN5A* gene expression, thus providing a new paradigm of *SCN5A* expression regulation that may shed new light into the understanding of cardiac disease.

1. Introduction

Sodium channel, voltage-gated, type V alpha subunit ($Na_V1.5$) drives the sodium current that initiates the upstroke of the cardiac action potential [1]. $Na_V1.5$ interacts with several regulatory proteins (β-subunits, Nedd4-2 ubiquitin ligase, calmodulin, among others) that modulate $Na_V1.5$ membrane trafficking and function [2]. Genetic mutations in the *SCN5A* gene, which encodes the $Na_V1.5$ subunit, have been linked to cardiac arrhythmias (Brugada syndrome, long QT syndrome type 3, idiopathic ventricular fibrillation, atrial fibrillation, progressive cardiac conduction defects, congenital sick sinus syndrome, and sudden infant death syndrome) [1,3].

However, recent findings also suggest that aberrant *SCN5A* gene expression may increase susceptibility to arrhythmogenic diseases. For example, low $Na_V1.5$ levels in heterozygous *Scn5a* +/− knockout mice recapitulate cardiac defects found in human individuals carrying disease-associated *SCN5A* mutations, and the severity of these defects is directly correlated with levels of $Na_V1.5$ expression [4]. In addition, certain haplotypes in the *SCN5A* promoter have been associated with aberrant *SCN5A* promoter activity and QRS duration on the electrocardiogram [5]. Finally, a common genetic variant (rs6801957) found in a distal *SCN5A* enhancer region has been associated with abnormal *SCN5A* expression and slow cardiac conductance [6,7]. The rs6801957 variant impairs T-box transcription factor 3 and 5 (TBX3/TBX5) binding

Abbreviations: ChIP, chromatin immunoprecipitation; cRNA, complementary RNA; ddPCR, droplet digital PCR; GATA-BS, GATA binding sites; HEK, human embryonic kidney; *Re*-ChIP, sequential ChIP; $Na_V1.5$, sodium channel, voltage-gated, type V alpha subunit; TSS, transcription start site; TF, transcription factor; WT, wild-type.

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to the enhancer, and has been proposed to promote changes in *SCN5A* expression. Collectively, these few studies suggest that dysregulation of *SCN5A* gene expression may be linked to cardiac disease, and that understanding the mechanisms of *SCN5A* transcriptional regulation may uncover novel determinants of sodium channel-related cardiac diseases.

The core promoter of the human *SCN5A* gene spans from nucleotides −261 to +140 relative to the transcription start site (TSS, +1) [8], and it contains conserved regions with putative regulatory functions. Downstream conserved regions may also include potential *cis*-regulatory elements, between TSS and exon 1 and within intron 1 [9]. In particular, intron 1 contains a predicted GATA1 binding site (BS). Mutation of this site reduces the promoter activity of the *Scn5a* gene in neonatal mouse cardiomyocytes, suggesting a direct role of GATA TFs in the regulation of basal *SCN5A* transcription [8]. The mammalian genome encodes six GATA TFs (GATA1–6). From those, GATA4, GATA5 and GATA6 are expressed in mesoderm- and endoderm-derived tissues, including the heart [10]. GATA-BS are present in *cis*-regulatory regions of multiple cardiac-specific genes and are required for their proper cardiac expression [11]. GATA4 is considered a master regulator of cardiac transcriptional networks, and plays a key role in cardiogenesis and in adult cardiac cells [12]. Mechanistically, GATA4 acts synergistically with other TFs and activates gene expression by promoting H3K27ac deposition [13]. Of note, GATA4 has been described to be involved in the developing atrioventricular cardiac conduction system; accordingly, heterozygous *Gata4* +/− mice display short PR intervals [14]. Mutations in the *GATA4* gene affecting DNA binding or interactions to other TFs have also been linked to heart dysfunction [15,16].

Here, we explored a potential role of GATA TFs on transcriptional regulation of the human *SCN5A* gene. We found that cardiac GATA TFs, mainly GATA4 and also GATA5, regulate the expression of the *SCN5A* gene via a synergistic mechanism. In fresh-frozen human heart samples, we observed that *GATA4* transcript levels positively correlate with *SCN5A* transcript levels. Overall, we suggest that GATA4 plays a major role in the regulation of the *SCN5A* gene in the human heart, which may shed new light into the understanding of human cardiac arrhythmias.

2. Materials and methods

2.1. Cells, antibodies and primers

Cardiac cells derived from embryonic rat ventricle (H9c2 cells) and human embryonic kidney 293 (HEK293) cells were maintained under standard cell culture conditions. Antibodies used in the experiments were: α-GATA4 (sc-1237x), α-GATA5 (sc-9054x), α-GATA6 (sc-7244x) (all from Santa Cruz Biotechnology, Dallas, TX, USA), α-HA (ab1424, Abcam, Cambridge, UK), α-actin (A2066, Sigma, St Louis, MO, USA), and α-FLAG (F1804, Sigma). Secondary HRP antibodies were: α-rabbit (32460), α-goat (31402), and α-mouse (32430) (all from Thermo Scientific, Rockford, IL, USA).

For qPCR gene expression analyses, we used the following rat primers: *Gata4* (QT02350684) and *Scn5a* (QT00186263) from Qiagen (Hilden, Germany); *Nppa* (R_Nppa_1) and *Kcnh2* (R_Kcnh2_1) from Sigma; *β-actin* (Fw 5′AGCCATGTACGTAGCCATCC′, Rv 5′ CTCTCAGCTGTGGTGGTGAA3′). For droplet digital PCR (ddPCR) analyses, we used the following validated expression probes: GATA4-FAM (dHsaCPE5050488), GATA5-FAM (dHsaCPE5036300), GATA6-FAM (dHsaCPE5037510), GATA4-HEX (dHsaCPE5050489), and GADPH-HEX (dHsaCPE5031597) from Bio-Rad (Hercules, CA, USA).

2.2. Plasmids and site directed mutagenesis

Details for plasmids and site directed mutagenesis are provided in Supplementary Materials and Methods.

2.3. Computational analyses

TFSEARCH and MEME bioinformatic tools were used to identify GATA-BS in the *SCN5A* proximal promoter (from positions - 1125 bp to +857 bp from the TSS).

2.4. Human cardiac tissue collection

Human left ventricle samples were collected from fourteen end-stage heart failure patients undergoing cardiac transplantation (Hospital Clínic, Barcelona). Control left ventricle heart samples, not used for transplantation, were obtained from four organ donors (Hospital Clínic, Barcelona). All individuals signed a written consent to participate in the study. Biopsies were collected from the explanted heart, washed and snap-frozen in liquid nitrogen immediately after surgery. To ensure minimum variation due to sample collection, all samples were dissected from the same heart area, the anterior wall of the left ventricle, by the same person. Histologically, samples comprise the three heart layers (epicardium, myocardium and endocardium). All procedures were approved by the ethical committees of the Hospital Dr. Josep Trueta de Girona and the Hospital Clínic de Barcelona and conform the principles outlined in the Declaration of Helsinki.

2.5. Chromatin immunoprecipitation (ChIP) and sequential chromatin immunoprecipitation (Re-ChIP) assays

ChIP and *Re*-ChIP assays were performed using human adult left ventricle samples. ChIP was adapted from Gomes et al., 2006 [17] and *Re*-ChIP from Furlan-Magaril et al., 2009 [18]. An expanded description of these techniques is available in Supplementary Materials and Methods.

2.6. Transient transfection and luciferase assays

PromoterA-luciferase or PromoterB-luciferase (200 ng), EF1αPromoter-Renilla (20 ng) constructs, and GATA expression vectors were transfected into H9c2 cells using Lipofectamine 2000 (Life Technologies, Grand Island, NY, USA) following the manufacturer's specifications. We transfected 1200 ng of each GATA expression vectors, except for the dose-response experiment in which we used 600, 1200 and 1800 ng. DNA for each condition was equalized with pcDNA3.1. Cells were harvested 48 h later with passive lysis buffer (Promega, Madison, Wi, USA), and processed for firefly and renilla luciferase activity with Dual Luciferase Reporter Assay System on a GloMax-96 luminometer (Promega).

2.7. RNAi experiments

We transfected H9c2 cells with 25 nM of ON-TARGETplus Non-targeting Pool (D-001810-10) or two different siGENOME siRNA Rat *Gata4* (D-090725-02 and D-090725-03; all from Dharmacon, Thermo Scientific) using Dharmaphect (Thermo Scientific). 48 h post-transfection, we isolated total RNA using the RNeasy Mini Kit (Qiagen) and treated the samples with DNase I to remove genomic DNA contamination. After reverse transcription of 1 μg of RNA (Reverse Transcription kit, Qiagen) we performed qPCR with validated primers for the target genes (*Gata4*, *Scn5a*, *Nppa*, *Kcnh2*, and *β-Actin*) and the Kapa-SYBR Green detection (Kapa Biosystems, Wilmington, MA, USA) on a Mastercycler ep realplex (Eppendorf, Hauppauge, NY, USA).

For the luciferase experiments, H9c2 cells were transfected with 25 nM of control or *Gata4* siRNAs and re-transfected after 24 h with PromoterA-luciferase or PromoterB-luciferase (200 ng) and EF1αPromoter-Renilla (20 ng) constructs. Cells were harvested 24 h later and processed for luciferase assay as described above.