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Induction of high STAT1 expression in transgenic mice with LQTS and heart failure

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Abstract

Cardiac-specific expression of the N1325S mutation of *SCN5A* in transgenic mouse hearts (TG-NS) resulted in long QT syndrome (LQTS), ventricular arrhythmias (VT), and heart failure. In this study we carried out oligonucleotide mircoarray analysis to identify genes that are differentially expressed in the TG-NS mouse hearts. We identified 33 genes in five different functional groups that showed differential expression. None of the 33 genes are ion channel genes. *STAT1*, which encodes a transcription factor involved in apoptosis and interferon response, showed the most significant difference of expression between TG-NS and control mice (a nearly 10-fold increase in expression, $P = 4 \times 10^{-6}$). The results were further confirmed by quantitative real-time PCR and Western blot analyses. Accordingly, many interferon response genes also showed differential expression in TG-NS hearts. This study represents the first microarray analysis for LQTS and implicates *STAT1* in the pathogenesis and progression of LQTS and heart failure.

Keywords: Cardiac sodium channel gene *SCN5A* mutation N1325S; Long QT syndrome (LQTS) and ventricular tachycardia; Microarray analysis; Dilated cardiomyopathy and heart failure; STAT1

The long QT syndrome (LQTS) is characterized by prolongation of the QT interval and T wave abnormalities on electrocardiograms (ECG) [1,2]. LQTS is associated with symptoms including syncope, seizures and sudden death caused by a specific ventricular arrhythmia, *torsade de pointes* [1,2]. One of the major genes identified for LQTS is the *SCN5A* gene on chromosome 3p21–23 (LQT3), which accounts for 10–20% LQTS cases [2,3]. *SCN5A* encodes a voltage-gated sodium channel $Na_v1.5$, which is mainly expressed in the heart and responsible for the generation and rapid propagation of electrical signals (action potentials) in cardiomyocytes [4,5]. Besides gain-of-function mutations associated with LQTS, loss of function mutations in *SCN5A* were demonstrated to be involved in the pathogenesis of both Brugada syndrome [6] and progressive cardiac conduction defects (PCCD) [7]. Mutations of *SCN5A* have also been reported to be involved in dilated cardiomyopathy/heart failure [8,9].

The N1325S mutation in *SCN5A* is a substitution of an asparagine residue by a serine at position 1325 in the intercellular region of domain III S4–S5 of $Na_v1.5$, and is one of the earliest mutations identified in LQT3 families [3]. It disrupts the Na^+ channel inactivation and generates the late persistent I_{Na} inward current. Overexpression of the N1325S mutation in *Xenopus* oocytes and HEK293 cells induced dispersed reopening in the late inactivation phase,

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which produced a late persistent inward sodium current [10,11]. We have expressed the *SCN5A* N1325S mutation in the mouse heart (TG-NS mice) [12]. The TG-NS transgenic mice showed prolongation of the QT interval on ECG and high incidences of spontaneous polymorphic VT followed by sudden cardiac death [12,13]. The electrophysiological studies of cardiomyocytes from the transgenic mice showed that the N1325S mutation produced a late sodium current and prolonged the cardiac action potential duration, which is expected to prolong the QT interval on ECG [12,14]. Recent studies also detected the phenotype of dilated cardiomyopathy and heart failure in TG-NS mice [15] as well as in a human patient with the N1325S mutation of *SCN5A* [14]. Age-dependent apoptosis and abnormal calcium handling were also demonstrated in the TG-NS cardiomyocytes, and are the likely causes of dilated cardiomyopathy and heart failure [15]. However, the molecular mechanism for cardiomyocyte apoptosis in TG-NS mice is not known. In this study we found that the expression of the *STAT1* gene was highly induced in TG-NS hearts, which may be a cause of apoptosis in these mice.

The *STAT1* gene encodes one of the signal transduction and activator of transcription factors (STATs) which are involved in transduction of signals from various ligands (cytokines, growth factors, stress-induced stimuli) to the nucleus through Janus tyrosine kinases (JAKs) or mitogen-activated protein (MAP) kinases [16]. Seven different STAT family members have been identified, which are activated by different cytokines [17]. *STAT1* mediates the response to interferon (IFN)-α and IFN-γ and has been shown to be pro-apoptotic [17]. STAT1-deficient mice are more susceptible to development of tumors, which implicates *STAT1* in oncogenesis [16]. No transgenic mice with over-expression of *STAT1* were developed, thus, the physiological effect for over-expression of *STAT1* is unknown.

Microarray analysis is an unbiased approach to study expression of thousands of genes simultaneously in a system. To date, no microarray analysis or other large-scale gene expression studies have been performed for LQTS, either in the humans or mice. Here, we took advantage of our mouse model for LQTS, the TG-NS mice, to explore global gene expression re-programming in these mice. We used mouse oligonucleotide microarrays with 22,690 unique genes to determine gene expression differences between TG-NS and non-transgenic control mice. A surprisingly large number of genes showed differential expression between the two types of mice, which may be partly caused by the marked up-regulation of transcription factor STAT1 as validated by RT-PCR and Western blot analyses. These results implicate *STAT1* in the pathogenesis and progression of LQTS and heart failure and offer insights into the observation of cardiomyocyte apoptosis in TG-NS mice.

Materials and methods

Transgenic mice. Human mutant *SCN5A* gene with the LQTS-causing mutation N1325S was expressed in the mouse heart using a cardiac specific promoter, the mouse α-myosin heavy chain (α-mMHC) promoter, and we named this line of transgenic mice as TG-NS. Transgenic mice with cardiac-specific expression of wild type *SCN5A*, TG-WT, were also lately created. The creation of TG-NS and TG-WT mice was reported by us previously [12,18], and they carry the comparable number of the transgenes and have a comparable level of *SCN5A* expression. Genotyping of the positive TG-NS mice was performed by polymerase chain reactions (PCR) using genomic DNA isolated from mouse tails/toes using the tail lysis buffer (50 mM Tris–HCl, 100 mM EDTA, 100 mM NaCl, 1% SDS). We used PCR primers 5′-TGT CCG GCG CTG TCC CTG CTG-3′ and 5′-CTC ATG CCC TCA AAT CGT GAC AGA-3′ for specific amplification of the *SCN5A* transgene and primers 5′-GGC ACC TGC TGC AAC GCT CTT T-3′ and 5′-GGT GGG CAC TGG AGT GGC AAC TT-3′ for amplification of *AGGF1* that serves as an internal control for quality of mouse genomic DNA. PCR was performed using standard procedures.

Microarray analysis. Total RNA was prepared from heart tissues of the non-transgenic control and TG-NS mice. First, heart tissues were homogenized by a polytron homogenizer (PT3100, Dispersing and Mixing Technology by Kinematica). Total RNA was then isolated using the TRIzol reagent (Invitrogen). The integrity and purity of the RNA was confirmed visually on a 1% denaturing agarose gel, and by measuring the optical density ratio (A260/A280). Double-stranded complementary DNA (ds-cDNA) was synthesized from 15 μg of total RNA using the Superscript Choice System (*Invitrogen*) with an HPLC-purified oligo-dT primer containing a T7 RNA polymerase promoter (GENSET, La Jolla, CA) as instructed by the manufacturer. The cDNA was extracted by the Phase Lock Gel (PLG) kit (Eppendorf) and purified by ethanol precipitation. *In vitro* transcription was performed with 1 μg of ds-cDNA using the ENZO BioArray RNA Transcript Labeling kit (ENZO Diagnostics). Fragmentation of biotinylated cRNA (20 μg), hybridization, washing, and staining were performed following the instructions by Affymetrix by the CWRU Gene Expression Core Facility. The Mouse Genome MOE430A arrays (Affymetrix) were used. Each array contains ~22,690 genes.

Statistical analysis. Microarray data was extracted from scanned images. GeneSpring 7.0 (Silicogenetics) was used to compare the data from three transgenic mice with those from three non-transgenic control littermates. All samples were considered as one group of replicates. The algorithm to generate a list of genes that showed a statistically significant difference between the two groups was described previously [19,20]. The median value for group comparisons was used. All raw data with a score less than zero were set to zero. Genes were further filtered by an absolute call: present (P) or marginally present (M) in the two groups for the up-regulated genes and down-regulated genes.

Quantitative real-time PCR (RT-PCR). Quantitative RT-PCR was performed using an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Total RNA was extracted from hearts using TRIzol (Invitrogen). Reverse transcription was performed with 5 μg of RNA using the Superscript Choice System (Invitrogen). Primers spanning exon-intron junctions were designed to avoid amplification of genomic DNA. PCR conditions were 50 °C for 2 min and 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Fluorescence changes were monitored with SYBR Green PCR Supermix (VWR) after every cycle, and melting curve analysis was performed at the end of 40 cycles to verify PCR product (0.5 °C/s increase from 55–99 °C with continuous fluorescence reading). The 18S gene was used to normalize samples for comparison. To quantify changes in gene expression, the $\Delta\Delta C_t$ method was used to calculate the relative fold changes as previously described [21].

Western blot analysis. To determine the expression level of the STAT1 protein, total proteins were extracted from mouse hearts. Hearts were homogenized with Polytron, and lysed on ice with the lysis buffer (0.5% NP-40, 20 mM Tris–HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA). The protein concentration was measured using the Bradford method (Bio-Rad). Equal amounts of protein extracts were separated on 10% SDS–polyacrylamide gels by electrophoresis. Western blot analysis was performed as described previously [5]. The blots were incubated with agitation at room temperature in the presence of a rabbit polyclonal anti–STAT1 antibody (Santa Cruz Biotechnology) (diluted in 1:500 in