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ZFHX3 knockdown increases arrhythmogenesis and dysregulates calcium homeostasis in HL-1 atrial myocytes

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ABSTRACT

Background: ZFHX3 plays an important role in the genesis of atrial fibrillation. However, the atrial electrophysiological effects of ZFHX3 are not clear. This study sought to investigate roles of ZFHX3 in atrial electrophysiology and calcium homeostasis by using HL-1 atrial myocytes knocked-down with ZFHX3.
Methods: Patch clamp, confocal fluorescence microscopy and Western blot were used to study electrical activity, ionic currents, calcium homeostasis and protein expressions in stable ZFHX3 shRNA cells.
Results: As compared to control, ZFHX3 shRNA cells with 28% decline of ZFHX3 protein had a larger sarcoplasmic reticulum Ca^{2+} content by 62%, Ca^{2+} transient by 20%, and calcium leak by 75%. ZFHX3 shRNA cells ($n = 35$) had shorter action potential duration (APD) at 50% (14.7 ± 0.9 versus 20.3 ± 1.4 ms, $P < 0.005$), and 20% (6.1 ± 0.3 versus 8.3 ± 0.8 ms, $P < 0.005$) repolarization than control cells ($n = 30$). ZFHX3 shRNA cells ($n = 10$) had larger amplitudes of isoproterenol (1 μM)-induced delayed afterdepolarization (14.1 ± 0.9 versus 7.2 ± 0.2 mV, $P < 0.05$) than control cells ($n = 10$). Besides, acetylcholine (3 μM) shortened APD at 90% repolarization to a greater extent (19 ± 4% versus 7 ± 2%, $P < 0.01$) in ZFHX3 shRNA cells ($n = 11$) than in control cells ($n = 12$). In addition, ZFHX3 shRNA cells had increased expressions of SERCA2a, ryanodine receptor, Kv1.4, Kv1.5 and Kir3.4. Moreover, ZFHX3 shRNA cells had a larger SERCA2a activity, ultra-rapid delayed rectifier potassium currents, transient outward currents and acetylcholine-sensitive potassium currents.
Conclusions: ZFHX3 knock-down in atrial myocytes dysregulated calcium homeostasis and increased atrial arrhythmogenesis, which may contribute to the occurrence of AF.

1. Introduction

Atrial fibrillation (AF) is the most commonly sustained arrhythmia, and can increase the incidences of heart failure, stroke, and mortality [1–3]. Genome-wide association studies (GWASs) provide the potential to study the molecular mechanisms of AF in human genomes [4,5]. GWASs have successfully identified some single-nucleotide polymorphisms (SNPs) in three loci for AF: *PITX2*, *ZFHX3*, and *KCNN3* [4–9]. The first locus is on chromosome 4q25 in *PITX2* which encodes the paired-like homeodomain transcription factor 2. *PITX2* plays a role in the formation of the pulmonary vein myocardium [10] and reducing Pitx2c expression promotes AF in mice models [11,12]. The second locus for AF is on chromosome 1q21 in KCNN3 which encodes the calcium-activated potassium channel protein, that plays an important role in determining the AP morphology [13,14]. However, the role of ZFHX3 in the pathophysiology of AF is not clear.

ZFHX3 is a tumor suppressor gene in multiple cancers and inhibits cell proliferation [15–20]. ZFHX3 was shown to suppress the activity of AT-rich elements that regulate the alpha-fetoprotein gene [21,22]. In HepG2 cells, over-expressed ZFHX3 was found to form complexes with protein inhibitor of activated Stat3 (PIAS3) and suppress signal transducer and activator of transcription 3 (STAT3) mediated signaling [23]. Increased expression of STAT3 was observed in pacing-induced sustained AF and down-regulation of ZFHX3 can activate STAT3 [24,25], which suggests that ZFHX3 may modulate atrial electrical activity and contribute to the genesis of AF. Calcium dysregulation plays a critical role in the pathophysiology of AF. In this study, we knocked down the ZFHX3 gene in HL-1 atrial myocytes to study the role of ZFHX3 in atrial electrophysiology and calcium homeostasis.

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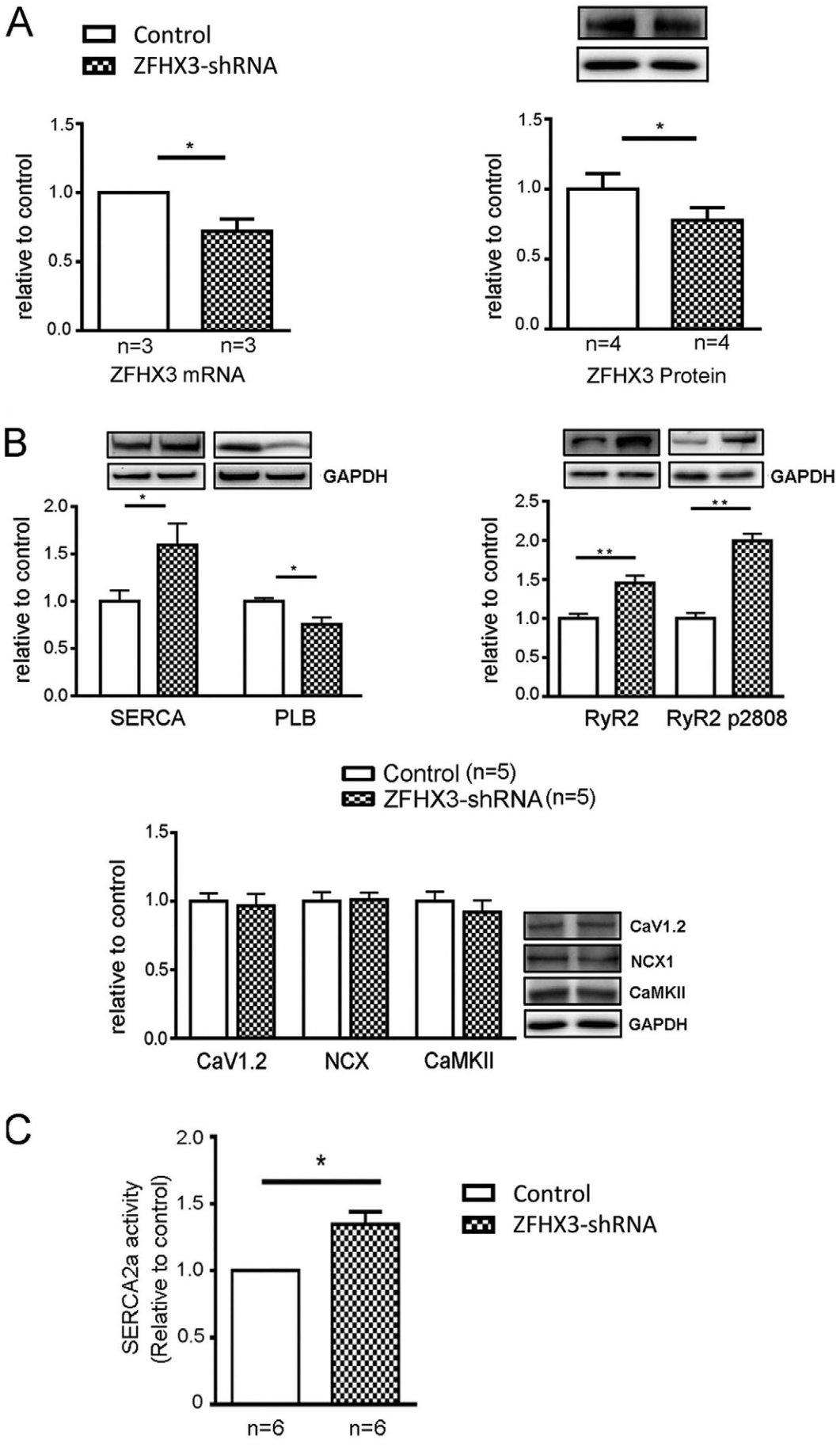
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Control
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relative to control
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1.0
0.5
0.0
*
n=3
n=3
ZFHX3 mRNA
*
n=4
n=4
ZFHX3 Protein
B
GAPDH
relative to control
2.0
1.5
1.0
0.5
0.0
*
*
SERCA
PLB
GAPDH
2.5
2.0
1.5
1.0
0.5
0.0
**
**
RyR2
RyR2 p2808
Control (n=5)
ZFHX3-shRNA (n=5)
relative to control
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1.0
0.5
0.0
CaV1.2
NCX
CaMKII
CaV1.2
NCX1
CaMKII
GAPDH
C
SERCA2a activity
(Relative to control)
2.0
1.5
1.0
0.5
0
*
n=6
n=6
Control
ZFHX3-shRNA