Presence and function of stress granules in atrial fibrillation protocol

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Abstract

Aims: Stress granules (SGs) are transient cytoplasmic mRNA and protein complexes that form in eukaryotic cells under stress. SGs are related to multiple diseases, but there are no reports of the existence of SGs in atrial fibrillation (AF); hence, the effects of SGs in AF are still unknown. Methods and results: Cell models of AF were established by field stimulation at 600 times per minute. HL-1 cells, cardiomyocytes and cardiac fibroblasts were transfected with pcDNA and G3bP1-cDNA plasmid by Lipofectamine 2000. The presence of SGs was detected by immunofluorescence staining against GTPase-activating protein SH3 domain binding protein 1 (G3BP1) and poly(A)-binding protein 1 (PABP-1) and electron microscopy. Stable HL-1 cell lines transfected with lentivirus overexpressing G3BP1 (pEGFP-LV-G3BP1) were constructed to further investigate the role of SGs in AF. The effects of SGs on reactive oxygen species (ROS) and calcium overload in tachypaced HL-1 cells were studied by flow cytometry. The effects of SGs on cardiac fibroblast proliferation and the protein expression level of collagen I/III and fibronectin-1 were also studied. Here, we first showed that SGs detected by G3BP1 and PABP-1 are present and markedly elevated in both tachypaced mouse cardiomyocytes and HL-1 atrial cells. The presence of SGs in tachypaced atrial myocytes was also confirmed by electron microscopy. SG formation in HL-1 cells with G3BP1 overexpression significantly inhibited the rapid pacing-induced increase in ROS level and attenuated calcium overload (all $P<0.05$). Furthermore, SGs inhibited cardiac fibroblast proliferation ($P<0.05$) and decreased the protein expression level of collagen I/III and fibronectin-1 in cardiac fibroblasts stimulated by angiotensin II (all $P<0.05$). Conclusions: SGs are rapidly induced and present in AF, and upregulation of SGs confers cytoprotection against oxidative stress, calcium overload and atrial fibrosis in AF.

Citation: Guo Dong,Fengying Liang,Chengcheng Wang,Wei Han Presence and function of stress granules in atrial fibrillation protocol. **protocols.io**

dx.doi.org/10.17504/protocols.io.sqvedw6

Published: 19 Aug 2018

Materials

anti-G3BP1and anti-PABP1 primary antibodies by Abcam

DCFH-DA, DAPI, Claycomb medium, fetal bovine serum by Sigma – Aldrich

Lipofectamine 2000 by Life Technologies

DMEM, Pancreatin by Gibco - Thermo Fischer

Protocol