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Dissertations-Kurzfassung

Esophageal cancer related gene-4 contributes to lipopolysaccharide induced ion channel dysfunction in human-induced pluripotent stem cell-derived cardiomyocytes

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Background: Previous studies have demonstrated that inflammation can cause arrhythmias. The esophageal cancer related gene-4 (ECRG4) is widely expressed in multiple tissues and can be downregulated after injury and infection. ECRG4 was also found to be downregulated in atrial fibrillation. However, experimental studies regarding roles of ECRG4 in inflammation induced ion channel dysfunctions and underlying mechanism in human cardiomyocytes are still lacking.
Objective: The study aimed to investigate possible roles and mechanisms of ECRG4 in lipopolysaccharide (LPS) induced ion channel dysfunctions in cardiomyocytes.
Methods: For the study, human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) were generated from three healthy donors. LPS was applied to challenge hiPSC-CMs to mimic inflammation in cells. Adenovirus expressing ECRG4 small RNA and adenovirus expressing ECRG4 full sequence were used to knockdown ECRG4 and overexpress ECRG4, respectively, in hiPSC-CMs. Patch-clamp, immunostaining, real time PCR and western blot analyses were carried out.
Results: LPS-challenge changed mRNA expression levels of multiple ion channels, but in channel current measurements, LPS failed to change the sodium channel current (I_{Na}), L-type calcium channel current ($I_{Ca\text{-}L}$), transient outward current (I_{to}), rapidly activating delayed rectifier current (I_{Kr}), slowly activating delayed rectifier channel current (I_{Ks}) or ATP-sensitive potassium channel current (I_{KATP}), indicating that LPS effect on the function of these channels is minor. However, the action potential duration (APD) was prolonged, I_{SK} (calcium-activated K channel current) was reduced and I_{NCX} (Na/Ca exchanger current) was enhanced by LPS, suggesting that LPS can cause electrical dysfunctions in cardiomyocytes via affecting SK channels and Na/Ca exchanger.
ECRG4 was detected in hiPSC-CMs and showed co-localization with TLR4 (a LPS receptor). LPS treatment increased ECRG4 expression in mRNA and protein level. After ECRG4 knockdown, the expression level of TLR4 and its associated genes, inflammatory cytokines and chemotactic cytokines were decreased. ECRG4 knock-down shortened APD via increasing I_{SK} and decreasing I_{NCX}. ECRG4 knock-down intercepted LPS effects on APD, I_{SK} and I_{NCX}. These results indicate that the effect of LPS on ion channels depends on ECRG4. Furthermore, a selective blocker of IKK2 and NFκB, which are important signaling factors in inflammation, could reverse the effects of ECRG4 overexpression on I_{SK} and I_{NCX}, suggesting that the effects of ECRG4 on I_{SK} and I_{NCX} were through IKK2 and NFκB signaling.
Conclusions: This study demonstrates that ECRG4 contributed to the LPS induced ion channel dysfunctions in hiPSC-CMs. ECRG4 may play a critical role in the pathogenesis of inflammatory cardiac diseases.