Voltage-Gated Channel Alpha Subunit 5 (SCN5A), the main alpha-subunit of the cardiac sodium channel isofrom. Our previous work demonstrated that SCN10Ashort, a small fragment comprising of the C-terminus of SCN10A, increased sodium current when co-expressed with SCN5A. In the present study we therefore explored SCN10Ashort as a novel gene therapy target.

**Objective:** To validate SCN10Ashort as a gene therapy target to increase the cardiac sodium current.

**Methods:** HEK cells with stable SCN5A expression were transfected with GFP or SCN10Ashort-FLAG-YFP and fixed for immunocytochemistry. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) were transduced with GFP and SCN10Ashort-YFP lentiviral vectors. Sodium current and APs were measured using patch-clamp methodology. For in vivo studies, GFP and SCN10Ashort-YFP were packaged in adeno-associated viral (AAV) vectors and these vectors were validated by immunocytochemistry. Next, AAV vectors were injected into the left ventricle free wall of adult mice. Two weeks after injection, mice were sacrificed and cardiomyocytes were isolated for patch-clamping.

**Results:** Immunocytochemistry on HEK cells showed membrane expression of SCN10Ashort, co-localized with SCN5A. In hiPSC-CMs, overexpression of SCN10Ashort leads to a significant increase in AP upstroke velocity (195.5 ± 29.1 vs 113 ± 12.4, p < 0.01), compared to the GFP group. Other AP parameters were not significantly affected. In vivo studies confirmed that overexpression of SCN10Ashort increases AP upstroke velocity (302.4 ± 17.5 vs 214.1 ± 8.8, p < 0.01 versus GFP group). AP duration at 20% repolarization (APD20) was reduced (2.8 ± 0.4 vs 1.3 ± 0.1, p < 0.05), but APD50 and APD90 were unchanged.

**Conclusion:** The robust increase in sodium current after in vivo gene transfer with SCN10Ashort carries substantial promise for novel gene therapy applications for inherited sodium channelopathies and acquired pacemaker and conduction system disorders.

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CRISPRi GENE MODULATION IN PRE-DIFFERENTIATED HIPSC-CMS COMBINED WITH ALL-OPTICAL ELECTROPHYSIOLOGY

Julie Han BA and Emilia Entcheva PhD

**Background:** Current models for cardiotoxicity screening are limited to only evaluate hERG inhibition for related prediction of QT prolongation and torsadogenic potential. More comprehensive *in vitro* cardiotoxicity assays are being proposed using human induced pluripotent stem cell-derived...