Abstract: 909

Massive expansion of native human atrial cardiomyocytes by immortogenetics for multiscale arrhythmia research

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Introduction
Preclinical cardiac research has been largely based on animal-derived cellular models, thereby hampering clinical translation. While upcoming human pluripotent stem cell technology seems to decrease this gap between bench and bedside, its complex/multi-step protocol to produce cardiac muscle cells, its required expertise, and its trouble to produce large numbers of phenotypically homogeneous cardiomyocytes so far has limited broad application.

Purpose
We aimed to immortalize native human atrial cardiomyocytes to produce natural and standardized lines of these cells by gaining full control over their proliferation and differentiation with a common cell culture agent.

Methods
Human fetal atria (gestational age 18 weeks) were dissociated and transduced with a lentiviral vector directing myocyte-specific and doxycycline-inducible expression of simian virus 40 large T antigen (here defined as immortogenetics). Addition of doxycycline to the culture medium pushed cardiomyocytes towards a proliferative phenotype. Sixty proliferating clones were isolated, expanded and screened for their cardiomyogenic differentiation capacity upon doxycycline removal. Selected clones were characterised using various molecular biological and electrophysiological assays.

Results
Upon doxycycline removal (i.e. under differentiation conditions), cells spontaneously reacquired a cardiomyocyte-like appearance as judged by phase-contrast microscopy and were observed contracting. Simultaneously, these cells stopped proliferating, which was accompanied by a drop in large T level, loss of Ki67 expression and the development of sarcomeres with striated a-actinin and troponin T staining patterns. These cells were tagged conditionally immortalised human atrial cardiomyocytes (hereinafter called hiAMs). Optical voltage mapping of these hiAM monolayers revealed excitable cells showing homogeneous spreading of action potentials at 12,5±0,9 cm/s following 1-Hz point stimulation, with a mean APD80 of 203±27 ms. Monolayers of hiAM cells could easily be created as big as 10cm² while continuing to display homogenous conduction throughout the culture. In response to the addition of the KATP/Kir6.x channel opener P1075, the APD80 could be shortened to 119±20 ms. Single-cell patch clamp recordings of a hiAM clone in current-clamp mode confirmed excitability with a resting membrane potential of -62,2±4,3 mV, peak potential of 39,4±3,9 mV and APD80 of 339±9 ms.

Conclusion
We have generated first-of-a-kind lines of natural human atrial cardiomyocytes through immortogenetics, allowing massive cell expansion under proliferation conditions and robust formation of cross-striated, contractile and excitable cardiomyocytes after differentiation. Thereby, a user-friendly, clinically-relevant and much-anticipated research model has been produced, which application could range from disease modelling to...
electrophysiological studies at single-cell level and up to large-scale monolayers.