Assessment of cardioprotective properties of FSTL1 in vitro using human iPSC-derived cardiomyocytes

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Background/Introduction: Treatment options for heart failure are limited and therefore regenerative approaches have become a major focus in cardiovascular research. Follistatin-like 1 (FSTL1) has shown to exhibit cardioprotective properties in vitro and in small animal studies.

Purpose: Since the effect of FSTL1 on human cardiomyocytes is still to be elucidated, we aimed to assess and quantify whether FSTL1 exhibits cardioprotective effects in vitro by monitoring protection from apoptosis and induction of proliferation in human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) in simulated ischemia/reperfusion injury.

Methods: Human iPSC-CMs were exposed to various concentrations of hypoglycosylated bFSTL1 (bacterial cell expressed) and hyperglycosylated mFSTL1 (mammalian cell expressed) for 72 hours to assess effects on proliferation. A TUNEL assay was performed to determine the optimal H2O2 concentration for simulated ischemia/reperfusion injury. iPS-CM were supplemented with various concentrations of either bFSTL1 or mFSTL1 in simulated ischemia/reperfusion injury assays and analyzed by immunocytochemistry.

Results: iPS-CM cultures showed gradually increasing cell numbers when supplemented with ascending concentrations (0 / 5 / 10 / 50 / 100 [ng/mL]) of bFSTL1 yet without statistical significance. In cultures exposed to mFSTL1, a concentration of 5 ng/mL was associated with a significant increase of cell number compared to control (P = 0.018). However, no further proliferative effect was observed with higher concentrations. Induction of apoptosis from applying various concentrations of H2O2 lead to 4.0% (25 µmol/L H2O2) to 18.8% (100 µmol/L H2O2) apoptotic cells (P = 0.012). From these optimization experiments 50 µmol/L H2O2 was concluded as the concentration that best simulated ischemia/reperfusion injury (10.2% apoptosis, 32% total cell loss). In the simulated ischemia/reperfusion model, FSTL1 significantly increased the ratio of Ki67+ cells. Application of bFSTL1 induced an upregulation of Ki67+ cells, however solely with a concentration of 100 ng/mL (P = 0.004 vs. control; P = 0.015 vs. 0 ng/mL; P = 0.005 vs. 10 ng/mL; P = 0.048 vs. 50 ng/mL). Applications of 50 ng/mL mFSTL1 (P = 0.013 vs. control; P = 0.032 vs. 0 ng/mL) and 100 ng/mL FSTL1 (P = 0.016 vs. control; P = 0.043 vs. 0 ng/mL) were also associated with a significantly higher amount of Ki67+ cells compared to controls and cultures not treated with FSTL1.

Conclusion(s): FSTL1 treatment induces proliferation and protection from apoptosis in human iPSC-CMs, regardless of the FSTL1 isoform used. Based on these results, further research on the mechanism of FSTL1 in human cardiac cells should be carried out before translation to large animal studies to establish safety and efficacy of FSTL1 in anticipation of potential clinical application.