Engineered heart muscle for modelling human cardiac disease

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Patient-specific induced pluripotent stem cells (iPS) offer great and novel experimental models for studying human cells and disease-specific phenotypes. In particular, they offer unprecedented opportunities to investigate human cardiomyocytes which are a difficult cell type to obtain from patients. We have developed an *in vitro* model of heart muscle (termed Engineered Heart Muscle, EHM) to study human heart muscle physiology and pathophysiology “in the dish”.

We have utilized this technology to identify disease phenotypes of cardiomyopathies with defined genetic background but also non-genetic cardiomyopathies. To model the latter, pathological catecholamine levels associated with heart failure were simulated by treating EHM with increasing concentrations of norepinephrine (NE, 0.001-1 μM) for 7 days. Pathological NE (>0.01 μM) concentrations led to decreased force of contraction, cardiomyocyte death, and cardiomyocyte hypertrophy. BNP release was increased and the inotropic response to isoprenaline blunted; both are classical clinical hallmarks of heart failure. Heart failure treatment was then tested by adding the β1-adrenoceptor antagonist metoprolol (5 μM) or the α-adrenoceptor antagonist phenoxybenzamin (5 μM) to maximal concentrations of norepinephrine (1 μM). Metoprolol reduced cardiomyocyte death, partially improved force of contraction and inotropic response to isoprenaline, without a significant effect on cellular hypertrophy. Phenoxybenzamine had a significant effect on cell size associated with partially improved force but no effect on inotropic response to isoprenaline.

As an example for a genetic cardiomyopathy we have studied hypertrophic cardiomyopathy associated with RAF1 mutations in Noonan syndrome. Noonan syndrome is classified as “RASopathy”, a group of congenital multi-organ syndromes characterized by mutations in the RAS/-MAP kinase pathway. 95% of patients with a gain of function mutation in RAF1 present with hypertrophic cardiomyopathy. Specific treatment options are not known. To study Noonan cardiomyopathy iPS cells were derived from patients with confirmed RAF1 mutation. Also, RAF1 mutations were introduced into an embryonic stem cell (ESC) line by Zinc finger nuclease technology to ensure an isogenic control line. Noonan EHM from both iPS and ESC displayed features of hypertrophic cardiomyopathy with a hypercontractile phenotype, increased calcium sensitivity, and cardiomyocyte hypertrophy. To test if overactivation of the RAF1-MAPK pathway is indeed causing the disease phenotype we applied a MEK (MAPK) inhibitor (U0126, 10 μM) for 7 days during EHM development. MEK inhibition normalized force, calcium sensitivity, and cell size thereby reversing the disease phenotype. This indicates that EHM may not only be used to investigate disease mechanisms but also to test potential therapeutic interventions *in vitro.*

In conclusion, EHM reproduce pathophysiology of human cardiac disease to a high degree and may therefore serve as important experimental tool to fill the gap between classical cell biology *in vitro* and physiological organ function *in vivo*.