Differentiation of iPS-derived cardiac mesoderm cells into cardiomyocytes using inducible shRNA technology

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Topic(s):
Basic Science - Cardiac Diseases: Gene Therapy, Cell Therapy

Citation:
Funding Acknowledgements:
BMBF funding (FKZ 13GW0098, FKZ 13GW0099) DFG funding through the BSRT GSC 203

Introduction: The development of robust and scalable cardiomyocyte differentiation protocols from human pluripotent stem cells (hPSCs) offers the opportunity to derive large number of autologous cardiomyocytes for cell therapy. However, human induced pluripotent stem cells-derived cardiomyocytes (hiPSCs-CM) do not proliferate, are susceptible to ischemia and poorly retained in the recipient myocardium. In addition, arrhythmias are a severe side effect of cell therapy with hiPSC-CM, probably due to their immature phenotype. Based on previous observations in our lab, we speculate that the heart environment might be an important factor to enable full maturation and in situ coupling of the implanted cells in vivo. We therefore speculate that transplantation of robust, proliferative, partially committed, non-tumorigenic cardiac mesoderm cells and subsequent differentiation in situ may overcome these limitations.

Purpose: We seek to engineer hiPSC-derived cardiac mesoderm cells for implantation in the failing myocardium that, once they have settled, consolidated, and proliferated in situ, can be selectively induced to mature into cardiomyocytes.

Methods: Wnt signalling induces mesoderm lineage differentiation of hiPSCs and inhibits further maturation towards CM. To induce Wnt signalling inhibition, we designed a customized biomimetic shRNA targeting the Wnt agonist β-catenin fused to a fluorescent reported protein controlled by a Tet-ON promoter. This transgene was transferred to the safe harbour locus AAWS1 of the OPTI-OX hiPS cell line constitutively expressing the reverse tetracycline transactivator protein (rtTA) in the ROSA26 locus. In this configuration, gene expression can be activated in trans upon doxycycline administration.

Results: We studied the kinetics of gene expression activation on the engineered undifferentiated hiPSCs and observed that a maximal reduction of 80-90% β-catenin correlating with reporter expression was achieved upon a 4-day treatment with doxycycline. Importantly, this activation could be fully reverted 6 days upon withdrawal. Furthermore, in vitro differentiation of cardiac mesoderm cells into cardiomyocytes was enhanced upon doxycycline administration as compared to the untreated cells as assessed by the upregulation of cardiomyocyte specific genes and the increased percentage of cTNT+ cells.

Conclusion: An inducible system for targeted β-catenin downregulation by shRNA allows for significant, sustained, and reversible Wnt signalling modulation, which is sufficient to induce cardiomyocyte differentiation of cardiac mesoderm cells in vitro. This technology may be implemented to differentiate the transplanted cells in the failing myocardium, opening new prospects to overcome the current limitations of hiPSC-based cardiac cell therapy.