Efficient multi-genetic modification of human ESC and iPS cells for cardiomyocytes enrichment and tracking

K. Schwanke1, S. Müller1, S. Merkert1, U. Martin1, R. Zweigerdt1

1LEBAO, Medical School Hannover, Hannover, Germany

Purpose: To date, the generation of stable transgenic hESC lines remains inefficient and transgenic hiPS lines allowing lineage-specific enrichment of progenies have not been reported. Herein, we describe a non-viral strategy for the efficient, parallel introduction of multiple-plasmid constructs into hESC / hiPS by means of a single drug resistance. This enables, for example, the antibiotic-based purification of cardiomyocytes (CMs) that also express several reporter genes facilitating donor cell monitoring, quantification, and eventually functional assessment in vivo.

Methods: Our method allows single cell dissociation of common hESC / hiPS cultures without limiting cell vitality. Co-introduction of multiple plasmids was established by optimized electroporation. Cells were seeded in feeder-free cultures to facilitate antibiotic-based clonal selection; colonies were individually expanded and tested for the presence of multiple transgenes.

Results: Two factors were critical for the generation of stable lines in only 12 days: high transduction efficiency (of up to 60%) and high cell vitality post transduction thereby supporting high colony recovery. On average, about 20 transgenic clones were reproducibly achieved from 1.5 million treated hESC or hiPS; about 25% of these clones carried multiple transgenes as demonstrated by PCR and functional assessment. Clones were also analysed for the expression of pluripotency markers and the capacity to differentiate into all germ-layers in vitro and in vivo. Successful enrichment of essentially pure CMs was established following cardiomyogenic differentiation of transgenic clones. We also report the current application of these CMs for the in vivo optimization of donor-cell survival in rodent hearts and the electrophysiological assessment of drug toxicity in vitro.

Conclusion: This is the first report of stable hiPS clones resulting in the purification of functional CMs and the parallel expression of additional marker genes. These clones were generated using a novel, fast, and highly efficient method of transgene introduction, which is of broad interest for numerous gain and loss of function applications in pluripotent stem cell research. Notably, stable multi-transgene expression was observed for at least 25 passages, the latest time point assessed. Thus, the method overcomes the common issue of vector silencing in pluripotent stem cells often associated with viral transduction methods.