Establishing a screening system in iPSC derived myotubes for imaging calcium dynamics

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Bachelor thesis, molecular life sciences, molecular bioanalytics

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ABSTRACT
Calcium (Ca²⁺) plays an essential role in muscle contraction. Ca²⁺ is released from the sarcoplasmic reticulum (SR) upon neuronal stimulation, which results in muscle contraction. Sarco/endoplasm reticulum calcium ATPase (SERCA) is responsible for transporting Ca²⁺ from the cytosol back into the SR. Dysfunction of SERCA is involved in the pathogenesis of several muscle diseases such as Brody myopathy, Duchenne muscular dystrophy (DMD) as well as heart failure. Therefore it exists a common interest in stimulating SERCA pharmacologically. Induced pluripotent stem cells (iPSC) opens up new possibilities since their pluripotency gives them the ability to differentiate to all cell types of the body including skeletal and heart muscle cells. Being ethically accepted and susceptible to genetic modifications makes iPSC cells a very compelling instrument. GCaMP is a genetically encoded calcium indicator (GECI), which can be used to image calcium dynamics in real time. GCaMP reporter gene can be used for compound screening by targeting transporters involved in Ca²⁺-flow.

The goal of this bachelor thesis was to establish a screening system in iPSC cell derived myotubes for imaging Ca²⁺ dynamics in real time in the SR. Indeed 16 clones could be isolated, 1 homo- and 4 heterozygous clones were characterized through PCR and FACS. Selected clones were then differentiated to myotubes.

Through Immunofluorescence (IF) GCaMP could be localized within iPSC cells as well as in myotubes. With an FDSS assay fluorescence was measured after stimulating the myotubes. However fluorescence signal was at the lower limit of detection.

INTRODUCTION
Since SERCA plays an important role in the pathogenesis of muscle diseases, observing and measuring influx and efflux of Ca²⁺ in the SR is a key aim of research and pharmacology. Goal of this bachelor thesis was to establish an iPS cell line, which can be used as a tool to measure Ca²⁺-flow of the SR in real time. For this a modified GCaMP expressing in the ER was aimed to be introduced into an iPS cell line. When differentiating iPS cells to myotubes, this tool would be very useful to screen for compounds which modify Ca²⁺ flux in skeletal muscle.

RESULTS
Around 100 clones resulted from the transfection with CAG-GCaMPPer-2A-Puro, from which 16 were picked. Since some clones did not continue to grow, only 5 clones were analyzed by PCR. Tab.1 provides an overview about the PCR results.

Characterization of clones through PCR

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<th>Clone/Cells</th>
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DIFFERENTIATION OF IPS DERIVED MYOTUBES

Differentiation of iPS cells to myotubes in vitro occurs over 4 stages (Fig. 1).

Localization of GCaMP within the cell
Since GCaMP was emitting GFP signal only very faintly, there was need to localize GCaMP in the cells to asses if GCaMP is expressed in the SR.

FDSS assay reveals correlating signal upon stimulation
In order to measure the response of GCaMP/Per upon stimulation, Ca²⁺ release from the SR, was stimulated by epibatidine (EPI). If GCaMP is expressed in the SR, one would expect a decreasing signal of GFP, since Ca²⁺ streams out of the SR upon stimulation. FDSS analysis revealed that indeed right at the point of injection, GFP signal decreased and comes back up within 1 minute. As predicted the amplitude of the signal was very low (Fig. 3).

CONCLUSION
GCaMP/Per seems not to be strong and is detectable by FDSS and fluorescence microscope only very weakly. Since GCaMP/Per is thought to be a screening tool for compounds on SR-membrane, this weakness of the signal is not optimal and would cause inconvenience for a screen as well as loss of sensitivity.

To determine successful if GCaMP/Per is located in the ER (in iPS) and SR (in myotubes), further analyses by immunofluorescence should be performed. Localization of the SR and GCaMP/Per could be shown by co staining GCaMP/Per with anti-GFP and the ER resp. SR.

REFERENCES