Modifications of stem cell-derived cardiomyocytes using non-integrating RNA: applications for optogenetics, siRNA knockdown and dominant negative expression of mutated transcripts

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BACKGROUND

Modification of protein expression in cells can be achieved on different levels either by stable integration of genetic material into the genome (directed or undirected homologues recombination, CRISPR, zinc finger nucleases, etc.), transient transfection of DNA vectors or episomal expression (e.g. baculovirus/BacMam) technologies.

Stable integration of genes requires modification of the genome and therefore requires further characterization of the cell genotype and phenotype. Episomal expression with BacMam constructs is dependent upon the use of HDAC inhibitors for stable production of the transcript. Non selective HDAC inhibitors are known cardiotoxicants and lead to hiPSC-derived cardiomyocyte (hiPSC-CM) cell death within a few days. Transient transfection is often hampered by low efficiency or by the resultant toxicity of the transfection reagents.
We have identified the first system for stem cell derived tissues which utilizes non-toxic and very high efficiency lipid-based transient RNA transfection technology, termed "Xpress. 4U". 100% transfection rate can be achieved by repetitive transfection. This mRNA methodology allows for non-integrating modification and high expression/titratable levels of the protein of interest.

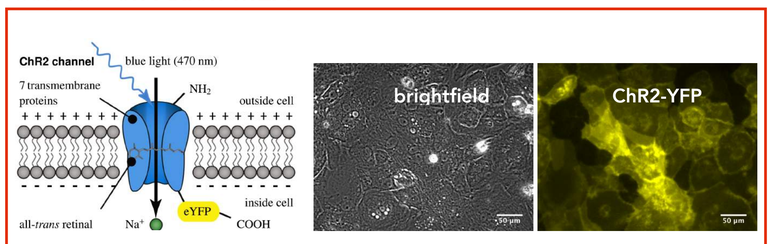
This opens up a wide breadth of applications including optogenetics, siRNA knock down of gene expression, over-expression of genes of interest and dominant negative expression of mutated proteins (i.e. disease modeling) without altering the host cell genotype. For these applications we provide proof of concept data within this presentation.

METHODS

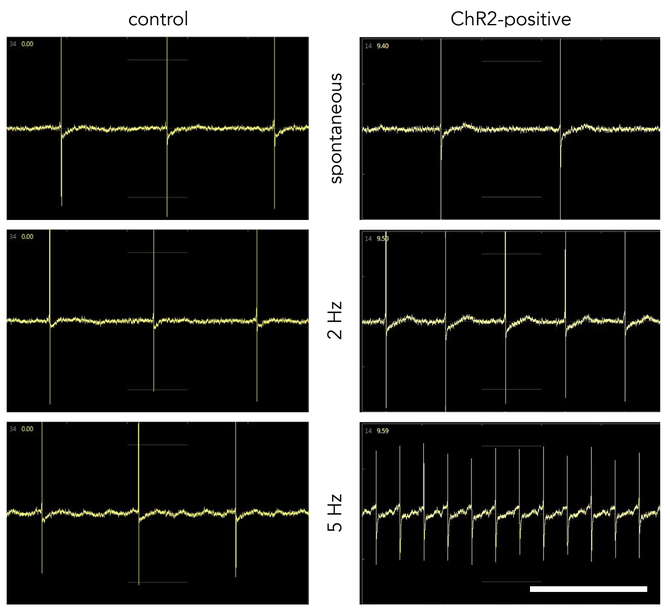
For optical stimulation experiments, Cor.4U® cells were cultured and transfected in T-flask format. Expression of ChR2-YFP fusion protein was detectable 17 hours post-transfection (hpt) and 10,000 cells per well were transferred to Axion Maestro microelectrode arrays.
For calcium Cor.4U® cells were transiently transfected with GCaMP6f in situ on a µClear 96-well plate and measured 24 hpt on a functional drug screening system. Prior to measurement, medium was replaced by Tyrode buffer containing 10 mM HEPES, 4.2 mM K^+ and cells were equilibrated to 37°C for 20 minutes. Sampling rate was 62.5 Hz with 16 ms of exposure time.
For knockdown experiments, Cor.4U® cells were cultured on T-25 flasks and transfected with eGFP mRNA or sequentially co-transfected with eGFP mRNA and Silencer eGFP siRNA (Ambion) for 10 minutes each, respectively. Prior to flow cytometry, phase contrast and fluorescence images of each culture were acquired 24 hpt using an epifluorescence microscope. Subsequently, cells were detached, collected and resuspended in PBS(-/-) & 2% FCS and passed through a flow cytometer.

RESULTS

Detection and optical pacing of ChR2-YFP expressing Cor.4U® on Axion-MEA

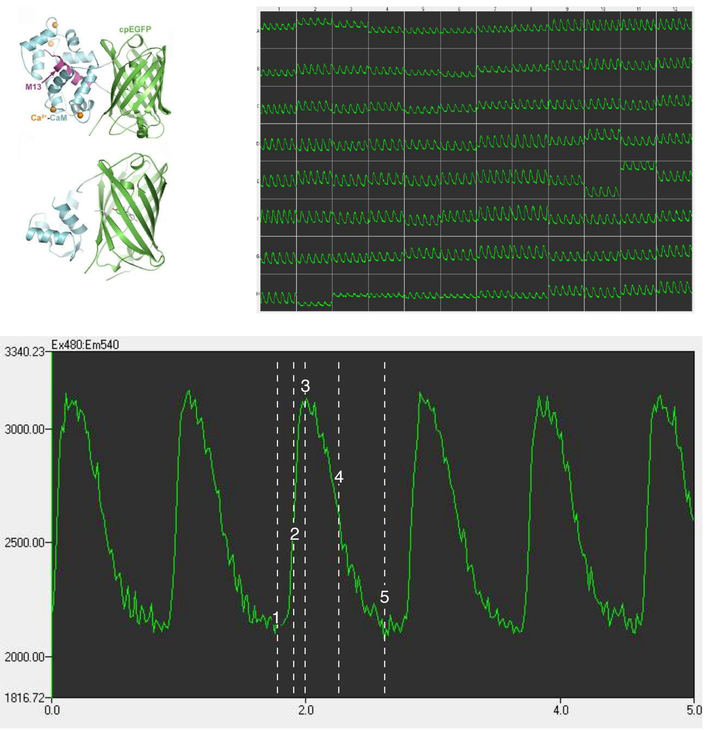


Structure and localization of ChR2-YFP fusion protein
Left: ChR2 is a light-gated cation channel that is activated by a conformational change of its retinal upon absorption of blue light (image: Standford University). **Right:** The C-terminal YFP-tag allows for detection of successful ChR2 expression in target cells (scale 50 µm).



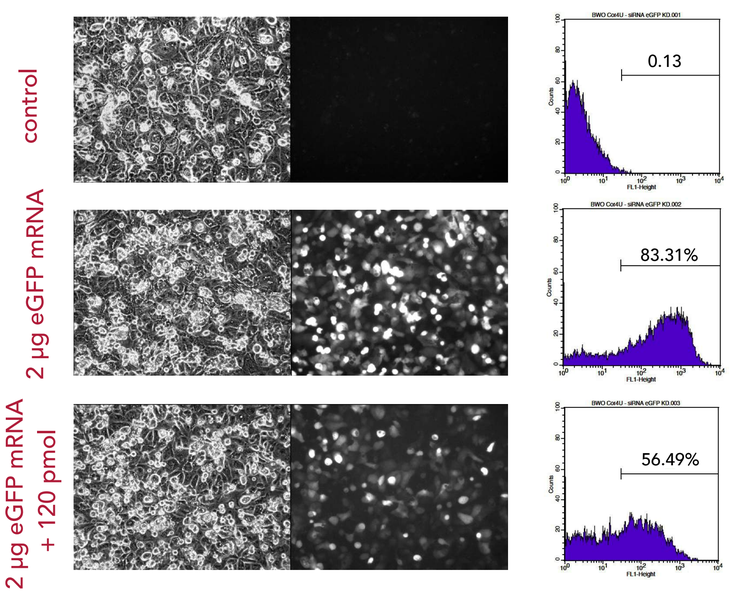
Performance of untreated and transfected Cor.4U® cells during optical stimulation
Control cells and transfected cells showed comparable beating frequencies before optical stimulation. Beating frequencies of ChR2 expressing cells followed optical stimulation with Lumos light delivery device up to 5 Hz (scale: 1 second)

Recording of calcium transients in GCaMP6f expressing Cor.4U® on Hamamatsu FDSS 7000EX



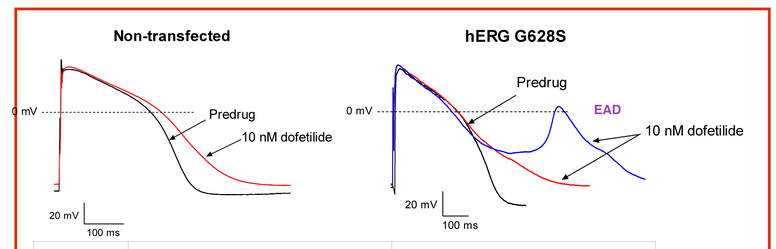
Measurement of GCaMP6f fluorescence intensity during changes in intracellular calcium concentrations in Cor.4U® cells
Top: simultaneous acquisition of fluorescence intensity in all 96 wells. Nearly all wells showed low background fluorescence and high signal to noise ratios. The duration of calcium transients varied between 550 - 600 ms with beat rates comparable to untreated cells (70 bpm). **Middle:** magnified fluorescence intensity profile of well C12. **Bottom:** Images 1 - 5 demonstrate high transfection efficiency of GCaMP6f and time course of fluorescence signal during cell contraction.

siRNA mediated knockdown of eGFP expression in Cor.4U®



Quantification of eGFP expression based on fluorescence intensity
Left: brightfield and fluorescence images of untreated (control), transfected and co-transfected Cor.4U cells. **Right:** flow cytometry data of respective cultures after microscopic image acquisition. eGFG transfected cells show high transfection efficiency after 10 minutes of incubation with transfection complexes. Co-transfected cultures show shift in fluorescence intensity in both microscopic images and flow cytometric analyses, confirming successful knockdown of eGFP expression.

Overexpression of hERG G628S (LQT2) in Cor.4U®



	Non-transfected			hERG G628S		
	Predrug	*10 nM dofetilide*	*% change*	*Predrug*	*10 nM dofetilide*	*% change*
MDP (mV)	-74±3	-64±5	-14 %	-73±2	-64±3	-13 %
APA (mV)	118±2	107±5	-9 %	104±4	96±5	-8 %
APD_{50} (ms)	279±20	303±22	+9%	288±17	357±37	+24%
APD_{90} (ms)	351±23	449±48	+28%	383±21	630±73	+65%

Perforated patch clamp of Cor.4U® cells overexpressing hERG G628S (LQT2). Transfected cells are more sensitive to dofetilide than non-transfected cells. APs are recorded from single cells using perforated patch clamp (gramicidin in the recording pipette) at 35±1°C. Stimulation frequency is 1 Hz. **Top left:** In non-transfected cells, dofetilide (specific hERG inhibitor) prolongs APD50 and APD90 (see table, n=6). In the presence of dofetilide, incomplete repolarization results in a depolarized Maximum Diastolic Potential (MDP) and shorter action potential amplitude (APA). No EADs were recorded. **Top right:** In cells transfected with G628S, APD50 and APD90 are somewhat longer than in non-transfected cells (see table, n=10). Like in non-transfected cells, MDP and APA are decreased. G628S cells were more sensitive to dofetilide than control cells. In dofetilide, APD90 and APD50 were increased by 65% and 24% respectively. EADs were recorded in over 50% of the G628S cells.

CONCLUSIONS

- isogenic control ⟹ quality control of cells possible before start of assay
- no need for viral vectors ⟹ non-integrative generation of desired phenotype
- ease of use, high efficiency, low cytotoxicity
- no interference with the electrical activity of the cells
- no chemical fluorescent dye required
- adaptable for siRNA mediated knockdown
- suitable platform for disease modeling (e.g. channelopathies)

COOPERATION PARTNER