A humanized phenotypic screening platform for chronic pain

Censo Biotechnologies and Cellectricon have joined forces to develop a first-in-kind humanized drug discovery platform to target chronic pain. The platform is based on human induced pluripotent stem cell–derived neurons in combination with Cellectricon's Cellaxess® Elektra screening system. The combination of highly functional screening with a human cell type of relevance for chronic pain has the potential to enable the generation of better drug candidates for this condition and other diseases.

Introduction

Physiologically relevant human models of chronic pain are essential for the development of new therapeutics and to overcome poor translation between animal studies and the clinical setting[1]. In this Application Note, we describe how sensory neurons derived from human induced pluripotent stem cells (hiPSCs) with a relevant expression profile can be applied in screening to identify compounds that change a disease phenotype, such as neuronal excitability, rather than the activity of specific targets. We envision that the described platform could be used in clinical trials in a dish[2], which could vastly improve the translation of drug candidates in human subjects with chronic pain and other devastating diseases.

Supply and differentiation of hiPSCs

HiPSCs were obtained from the European bank for induced pluripotent stem cells (EBiSC), and working banks of hiPSCs were produced. Each bank was then characterized by flow cytometry and morphology.

Differentiation was achieved in the Censo laboratories in Cambridge, UK, with a sensory neuronal differentiation protocol based on a publication by Young *et al.*[3]. In summary, fully characterized hiPSCs were seeded as single cells and at low confluence onto Matrigel. Induction of differentiation was achieved by dual SMAD inhibition with the small molecules SB431542 and LDN193189, followed by further neural commitment and patterning using DAPT, SU5402 and CHIR99021. Neural crest cells were cryopreserved, and quality control was performed to determine the quality and yield of neuronal cultures. Neural crest cells were matured into functioning neurons by the use of growth factors to facilitate sensory neuronal development.

Neuronal characterization

Each cryopreserved batch of sensory neurons was quality controlled at the neural crest and sensory neuronal development stages. **Figure 1** shows the typical quality control process carried out for each batch of hiPSC-derived sensory neuronal cells.

Immunocytochemistry was used to determine the protein-level expression of the peripheral nervous system marker SOX10 and the sensory neuronal marker BRN3A at day 46. At the gene-expression level, a panel of neural crest and sensory neuronal markers was used that included genes encoding developmental transcription factors such as *NEUROG1*, *OTX2*, *TFAP2B*, *POU4F1* and *ISL1*. Furthermore, expression of genes encoding late sensory neuronal markers and important sensory neuronal targets was also measured. These genes included *RUNX1*, *SCN9A*, *SCN10A*, *NTRK1* and *TRPV1*.

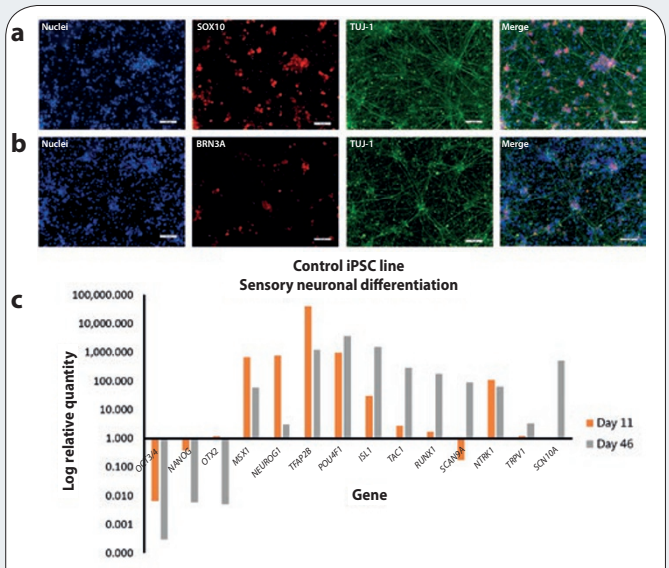

Figure 1 | Typical quality control process for each batch of hiPSC-derived sensory neurons. (**a**–**c**) Day 46 neurons express peripheral nervous system markers at the protein (**a**,**b**) and mRNA levels (**c**). Scale bars, 100 µm.

Daniel Tams[1], Paul Karila[2] & Ashley Barnes[1]

[1]Censo Biotechnologies Ltd., Babraham Research Campus, Cambridge, UK. [2]Cellectricon AB, Mölndal, Sweden. Correspondence should be addressed to A.B. (ashley.barnes@censobio.com).

APPLICATION NOTES

Quality control assessment, batch-to-batch consistency and assay reproducibility are all requirements for this platform technology, and for robust data generation.

High-quality cryopreserved and quality controlled sensory neuronal progenitor batches were provided to Cellectricon for the next stage of the process. The final stages of neuronal maturation were completed within the Cellectricon laboratories.

Functional drug screening in hiPSC-derived sensory neurons

The Cellaxess Elektra system enables electric field stimulation (EFS) and monitoring of neuronal cell cultures directly in 384-well microplates. Calcium imaging is used to monitor action potentials in cells during EFS while a homogeneous electrical field is applied. (**Fig 2a**).

The simultaneous EFS and imaging of the entire plate enables the identification of test compounds that modulate neuronal excitability in a high-capacity fashion while delivering electrophysiology-like accuracy. Physiological relevance is demonstrated by appropriate activity of clinically relevant tool compounds representing a wide range of mechanisms for chronic pain (**Fig. 2b** and **Table 1**).

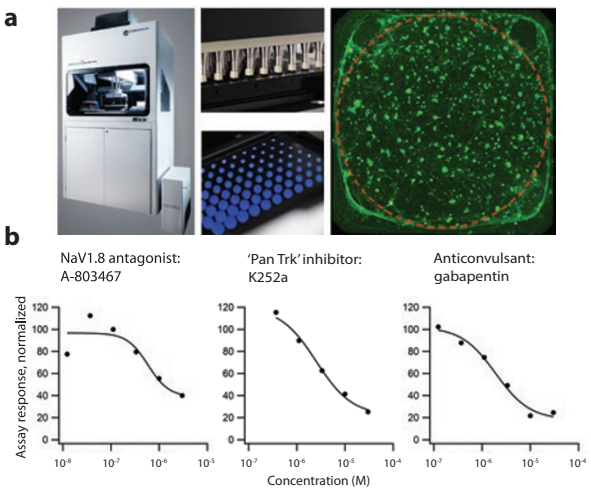

Figure 2 | Overview of the screening platform and the pharmacological data obtained from hiPSC-derived sensory neurons. (**a**) The Cellaxess Elektra system. Neuronal excitability is analyzed on the basis of the EFS-induced calcium transients. The red dashed circle indicates the area of the hiPSC cell mat affected by the electric field. (**b**) The concentration response curves show example mechanisms that are active in Censo's hiPSC-derived sensory neurons on the Cellaxess Elektra system.

Where applicable, the potencies align with effective plasma concentrations in chronic pain conditions[4,5]. Some variation was observed between lines from two individuals/assay replicates. It is therefore important to consider how many individuals need to be included in a screening strategy.

Assay capacity

A major benefit of using hiPSC-derived neurons is that cell supply will no longer limit the throughput of the system, as is the case when using primary tissue. The assay presented here is fit for screening libraries of up to 50,000 compounds in a relatively short time frame and is accordingly suitable for supporting medium-throughput drug discovery campaigns.

Table 1 | Effects of clinical and tool compounds investigated in two healthy control lines, showing that multiple clinically relevant mechanisms are active in Censo's hiPSC-derived sensory neurons on the Cellaxess Elektra system

Mechanism	Compound	IC_{50} (M)
Pan NaV blocker	Tetrodotoxin	2.9×10^{-9}
Pan NaV blocker	Tetracaine	2.7×10^{-6}
Pan NaV blocker	Mexiletine	1.7×10^{-5}
NaV1.7 blocker	PF-05089771	3.5×10^{-8}
NaV1.8 blocker	A-803467	2.9×10^{-7}
NaV1.8 blocker	PF-04885614	1.3×10^{-8}
Anticonvulsant; α2δ ligand	Gabapentin	4.9×10^{-7}
CaV blocker	Mibefradil	7.1×10^{-7}
HCN blocker	Cilobradine	1.2×10^{-6}
Promiscuous MOR agonist	Loperamide	6.6×10^{-7}
NSAID	Naproxen	9.8×10^{-7}
K channel opener	Flupirtine	Inactive
Pan Trk inhibitor	K252a	1.2×10^{-7}

Summary and conclusions

The data presented here show that that Cellectricon's Cellaxess Elektra system, when used in combination with hiPSC-derived sensory neurons produced by Censo, is well suited to investigate compound effects on neuronal excitability, with high relevance to human pain conditions. Because of the high capacity of the platform, we can generate highly refined compounds ready for clinical testing from medium-throughput compound libraries. We are now pleased to offer fully supported access to this first-in-kind humanized screening platform with the aim of identifying novel lead compounds with great potential for translation into new treatments for chronic pain.

Importantly, this platform is not limited to chronic pain. Censo provides cell-based models of disease using cells from a diverse range of tissue donors, and is specializing in the production of customer-specific or customer-generated hiPSC lines from large cohorts of individuals to create virtually any cell in the human body. Accordingly, Censo can provide hiPSC lines to support the development of new targeted treatments for disease in several therapeutic areas.

Moving forward, we also see potential for the use of this combination of technologies for 'clinical trials in a dish', which could vastly improve the translation of drug candidates in human subjects with chronic pain, neurodegenerative disorders and other devastating diseases.

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