SCALABLE DIFFERENTIATION OF HUMAN iPSC-DERIVED PSEUDO-UNIPOLAR NOCICEPTORS WITH IN VIVO-LIKE PROPERTIES AND TRANSLATIONAL APPLICABILITY

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Abstract

Development of new non-addictive pain medications requires advanced strategies to differentiate human induced pluripotent stem cells (iPSCs) into relevant cell types amenable for disease modeling and drug discovery. Here, we devised a highly efficient and scalable protocol that differentiates iPSCs exclusively into nociceptors under chemically defined conditions. By manipulating developmental pathways using small molecules, iPSCs were first converted into SOX10+ neural crest cells followed by differentiation into *bona fide* pseudo-unipolar BRN3A+ nociceptors. Detailed molecular and cellular characterization confirmed that differentiated nociceptors expressed typical neuronal markers, transcription factors, neuropeptides and over 150 ion channels and receptors. Focusing on pain-relevant receptors and channels expressed by iPSC-derived nociceptors (e.g. P2RX3, TRPV1, NAV1.7, NAV1.8), we demonstrated robust functional activities and differential response to noxious stimuli and specific drugs and demonstrate suitability for phenotypic screens. Lastly, a robotic cell culture system was used to automate the production of billions of cryopreservable cells for high-throughput drug screening, urgently needed to develop new nociceptor-selective analgesics and help to tackle the opioid crisis.

Materials and Methods

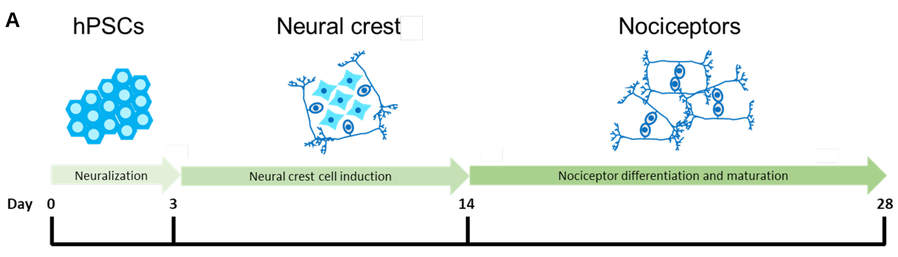

Fig. 1: Automated step-wise nociceptor differentiation. A. Controlled differentiation of hPSCs into functional nociceptors using small molecules and chemically defined conditions. **B.** Differentiation protocol was adapted to an automated cell culture system enabling standardized and scalable production of nociceptors from up to 90 iPSC lines in parallel.

Results

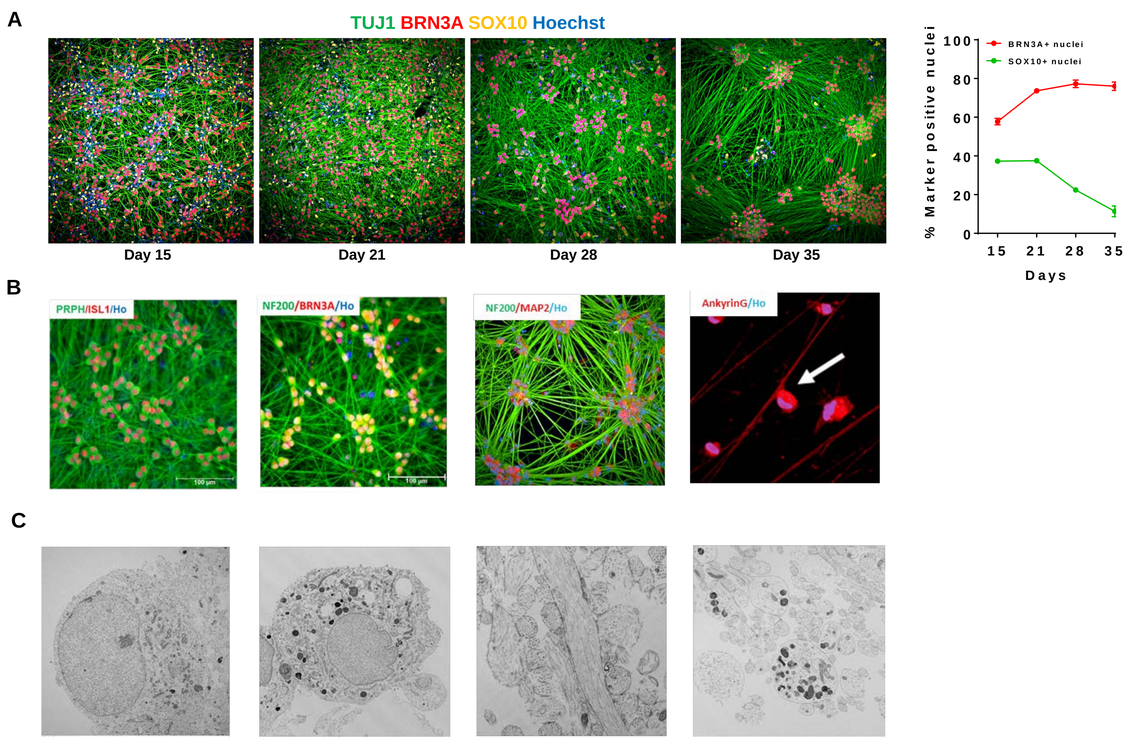

Fig. 2: Characterization of hPSCs-derived nociceptors. A. Quantification of immunolabeled cells at different developmental stages demonstrate the efficiency of the nociceptor differentiation protocol. **B.** Immunostainings showing that nociceptors express typical markers after 4 weeks of differentiation. Note the absence of MAP2+ dendrites and axon splitting labeled by AnkyrinG+ immunoreactivity. **C.** Transmission electron microscopic images show cell bodies and axons of peptidergic and non-peptidergic nociceptors, whereas dendritic structures are absent in these cultures.

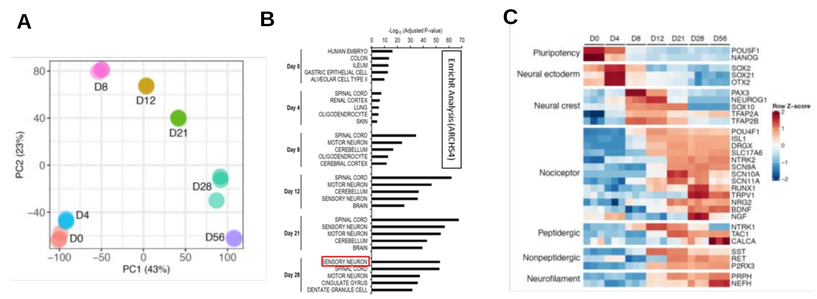

Fig. 3: Gene expression profiling of differentiating nociceptors (RNA-seq). A. PCA plot showing distinct signatures at different time points during nociceptor differentiation. **B.** EnrichR analysis (Lachmann et al., 2018) comparing the transcriptome of nociceptors at different differentiation stages to that of human tissue samples in the ARCH4 database. **C.** Heatmap illustrating the time-course and stepwise differentiation of hPSCs into neural crest and nociceptors.

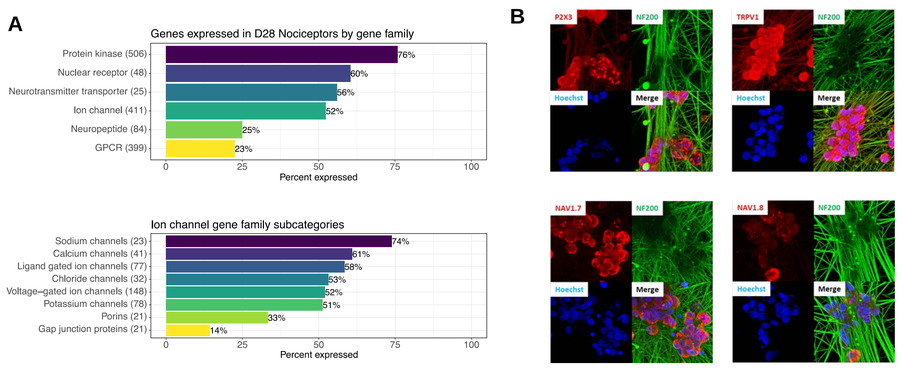

Fig. 4: Ion channels and receptors expressed by hPSC-derived nociceptors **A.** RNA-seq experiment revealed expression of 152 ion channels/receptors that can be classified into different gene families. **B.** Examples of immunocytochemical analyses showing expression of P2X3, TRPV1, NAV1.7 and NAV1.8.

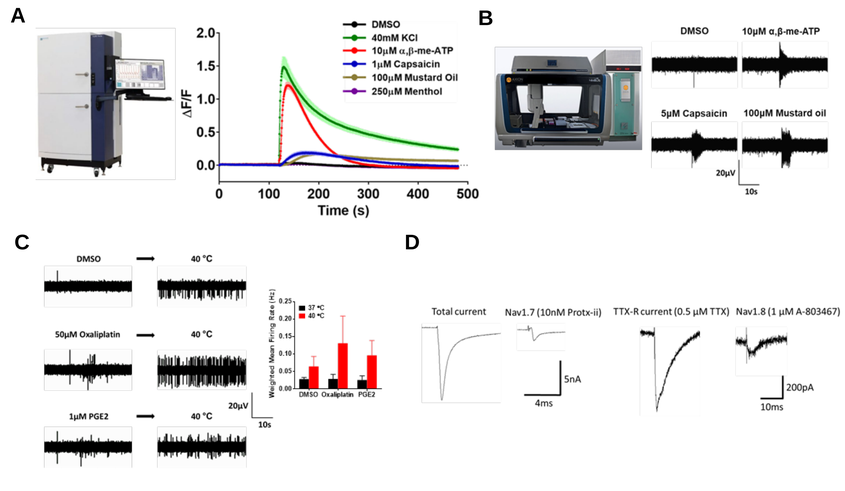

Fig. 5: Functional analysis of hPSC-derived nociceptors. **A.** FLIPR calcium imaging showing differential response of nociceptors to various stimuli. **B.** APEX robotic multi-electrode array (MEA) analysis demonstrates response to various stimuli known to activate nociceptors. **C.** MEA analysis indicates sensitization of nociceptors by oxaliplatin and PGE2 and their subsequent activation by temperature increase from 37 to 40 °C. **D.** Patch-clamp experiments and pharmacological isolation of NAV1.7 and NAV1.8 currents.

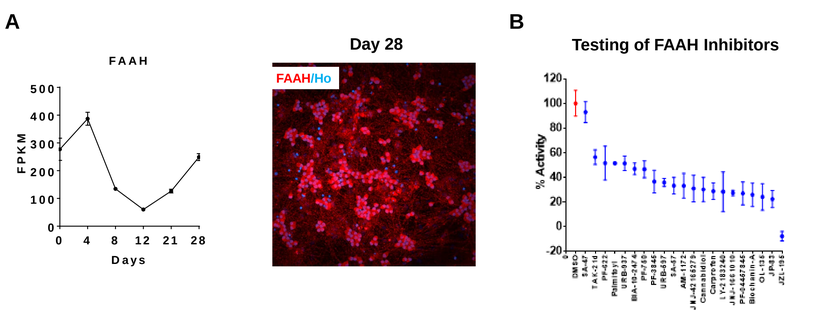

Fig. 6: Enzyme activity assay to demonstrate the utility of hPSCs-derived nociceptors for drug testing. **A.** RNA-seq of differentiating cells (day 0-28) and immunostaining show expression of FAAH (fatty acid amide hydrolase) by human nociceptors. **B.** Enzymatic assay (Ramarao et al., 2005) demonstrates inhibition of FAAH activity in nociceptors. Note the differential inhibitory potency of several known FAAH inhibitors and JZL-195 being the most potent inhibitor.

Summary

1. We successfully established a highly efficient, scalable protocol to differentiate hPSCs into functional nociceptors under chemically defined conditions.
2. We adapted the protocol to an automatic cell culture system to standardize and scale-up the production of nociceptors.
3. Human PSCs-derived nociceptors express typical markers, ion channels, neuropeptides, and display electrical activity that can be modulated by various stimuli.
4. Well-characterized human nociceptors will play key roles in disease modeling, pain research, and drug development.

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