Bulk and Single Cell Transcriptomic Analysis of Human iPSC-derived Sensory Neurons as a Model for Non-Opioid Pain Therapeutic Drug Discovery

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ISSCR 2025 HONG KONG

Introduction

With the recent FDA approval of Suzetrigine, Vertex's selective pain inhibitor targeting NaV1.8 sodium channels, there is renewed biopharma focus on non-opioid based pain drugs. The desire to improve predictability is fueling interest in new human-relevant models, with a strong focus on induced pluripotent stem cell (iPSC)-derived peripheral sensory neurons. These cells have the potential to advance pain research, however, a deeper understanding of how they recapitulate relevant expression and function of non-opioid pain targets is needed. In this study, baseline transcriptomic characterization of a novel human iPSC-derived sensory neurons, iCell® Sensory Neurons (FUJIFILM Cellular Dynamics), was performed and compared to human dorsal root ganglion (hDRG) and alternative published iPSC-derived sensory neuron protocols. The bulk RNAseq data demonstrated that iPSC-based methods yielded cells that clustered distinctly from hDRG. Within this grouping of iPSC-derived sensory neurons, subclusters were identified, suggesting that variations in differentiation protocols can impact the resulting sensory neuron populations. Importantly, iCell Sensory Neurons showed the high expression of non-opioid pain-relevant genes (i.e., SCN9A, SCN10A, TRPV1, PEIZO2, and P2RX3) which was corroborated with single cell RNA-seq data showing these genes were expressed across the sensorineural population. In addition, these sensory neurons also displayed accentuated expression of neural maturation markers, calcium handing genes, sodium channels, and potassium channels, as compared to cells from other iPSC differentiation protocols. These genomic data correlate with functional data in cell-based assays, including robust calcium influx responses to sensory-specific molecules (i.e., capsaicin). In addition, the genetic profile of iCell Sensory Neurons shows alignment with trigeminal ganglion markers, which aligns with the ability of the cells to secrete CGRP and show SNAP25 cleavage in response to Botulinum toxin. In summary, iPSC-derived sensory neurons have a transcriptome profile relevant for human in vitro modeling of pain and neuropathy. Importantly, iCell Sensory Neurons express premier non-opioid pain targets within a population that is enriched in ion channels and intracellular signaling pathways that correlate with advanced sensory function.

Methods

iCell® Sensory Neurons			
Catalog #	Donor	Sex	Vial Size
C1259	01279	Male	6M
C1260	01279	Male	1M
C1261	21527	Female	6M
C1262	21527	Female	1M

iCell® Sensory Neurons were thawed and maintained in culture using iCell Sensory Neurons Medium. Cells were evaluated at various timepoints for gene expression, immunocytochemistry and morphology. For bulk RNA sequencing, cells were lysed in RTL lysis buffer (QIAGEN) and total RNA was extracted (QIASymphony, QIAGEN). Total RNA sequencing was performed by Novogene. Cells were prepared for single cell RNA sequencing by following a slightly modified version of the fixed tissue protocol from 10X genomics (CG000553). Briefly, cells were lifted off the plate with gentle swirling in dPBS, then fixed in PFA containing buffer overnight. Cells were then digested to a single cell suspension with papain (Worthington), washed several times in quenching buffer to remove debris and frozen in storage buffer. Samples were stored at –80°C until sequencing which was performed by Washington University's Genome Technology Access Center. Comparative hiPSC-derived sensory neurons and hDRG RNASeq datasets were downloaded from Gene Expression Omnibus (GEO) database. For hiPSC-derived sensory neurons , the raw reads were downloaded from GEO (Deng 2024: PRJNA783035, Plumbly 2022: GSE187345, Zeidler 2021: GSE161530. Truong 2024: GSE275412) and processed through the FCEI bulk RNASeq pipeline (HiSat2/FeatureCount) to create TPM/FPKM files. For hDRG RNASeq, the Quantile-normalized TPMs file was downloaded and compared with our Quantile-normalized TPMs data. Functional assays using iPSC-derived sensory neurons were performed at 2-4 weeks in culture. Calcium assays were performed following iCell Lab Note Calcium Influx instructions and recorded using Hamamatsu FDSS µCell.

Morphology and Purity of iCell® Sensory Neurons

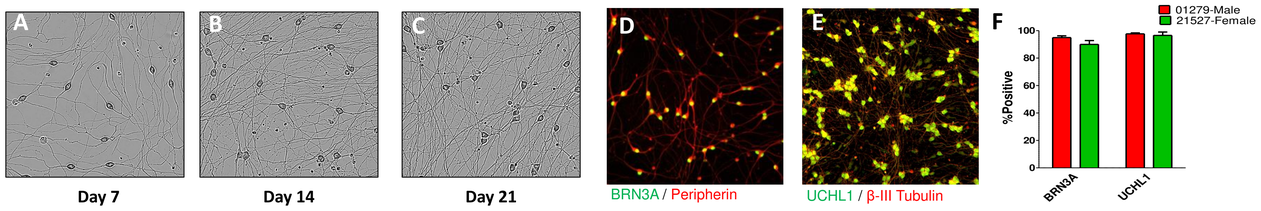


Figure 1. Brightfield morphology and purity by ICC. (A-C) iCell Sensory Neurons were plated onto Geltrex-coated 6-well plate (1x10^6 cells/well). Sensory neurons mature with a bright cell body, extensive neurite networks, and pseudo-unipolar morphology. ICC staining showed majority of the culture expressed sensory neuron markers Brn3A and peripherin **(D)**, and pan-neuronal markers UCHL1 and β-III Tubulin **(E)**. **(F)** Quantification of % positive marker expression across 4-6 lots each donor.

Comparison of iPSC-derived Sensory Neurons to Human DRG

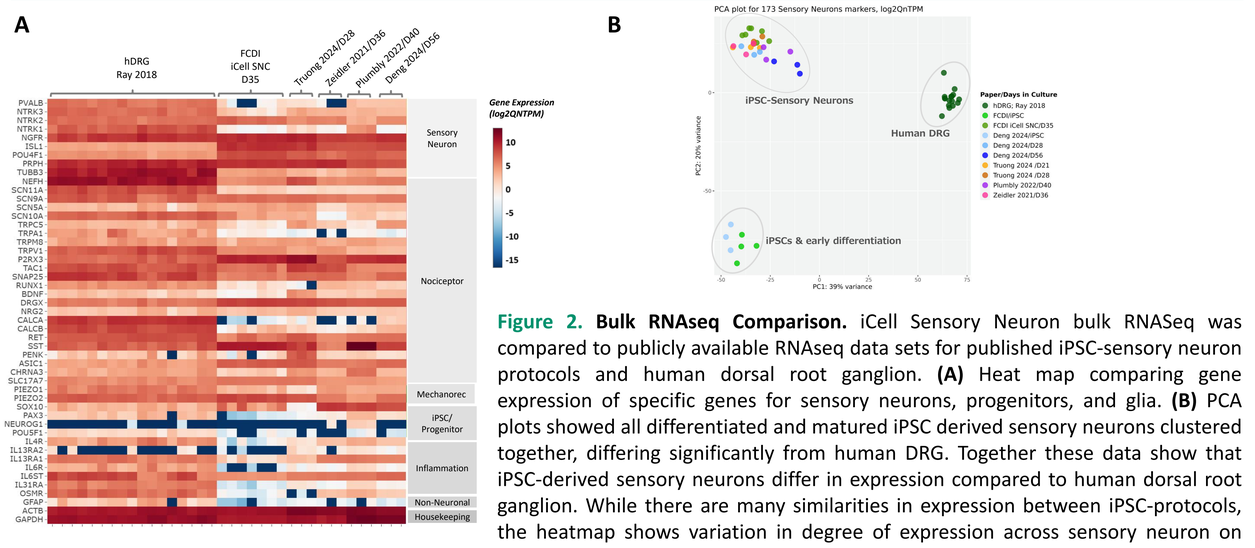
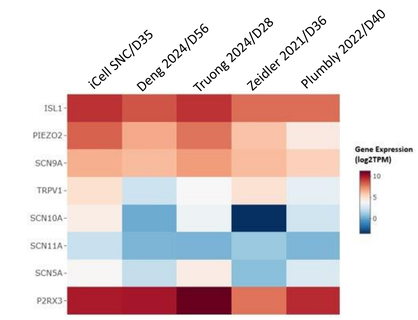
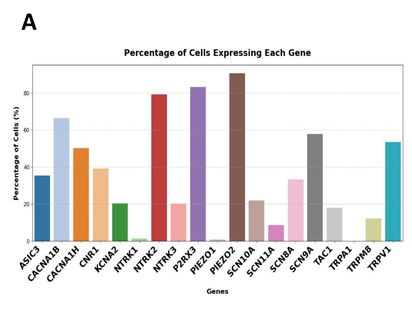


Figure 2. Bulk RNAseq Comparison. iCell Sensory Neuron bulk RNASeq was compared to publicly available RNAseq data sets for published iPSC-sensory neuron protocols and human dorsal root ganglion. **(A)** Heat map comparing gene expression of specific genes for sensory neurons, progenitors, and glia. **(B)** PCA plots showed all differentiated and matured iPSC derived sensory neurons clustered together, differing significantly from human DRG. Together these data show that iPSC-derived sensory neurons differ in expression compared to human dorsal root ganglion. While there are many similarities in expression between iPSC-protocols, the heatmap shows variation in degree of expression across sensory neuron on progenitor genes.

Expression of Non-Opioid Pain Targets and Nociceptor Genes





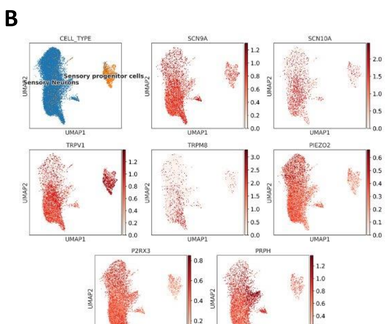
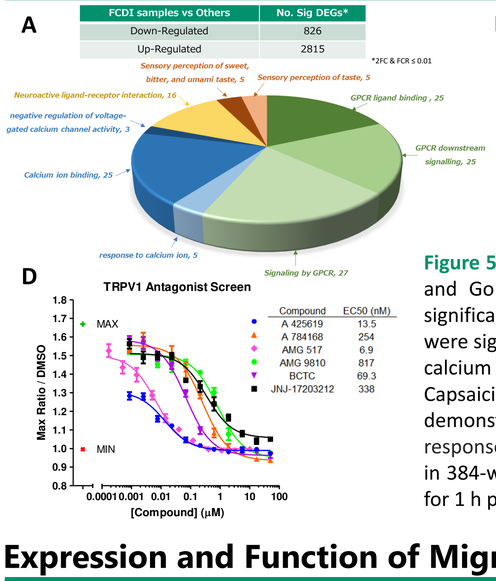
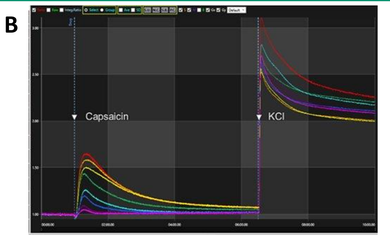


Figure 3. iCell Sensory Neurons Show High Expression of Non-opioid Pain Targets. Bulk RNAseq analysis was performed on key non-opioid and sensory neuron targets across iPSC-derived sensory neuron protocols. These data show variation of these marker expressions across iPSC-derived sensory neurons, with most cells expressing key pain targets to some degree. Importantly, iCell Sensory Neurons display an enhanced expression of TRPV1 and SCN10A, both genes important for nociceptor function and active targets for non-opioid pain research.

Figure 4. Single Cell RNASeq Analysis. iCell Sensory Neurons from the 21527 donor (female) were analyzed for single cell gene expression across two manufactured lots. **(A)** Bar graph showing % of cells expressing each gene within the entire population sample. Notedly, close to 60% of the population expressed TRPV1 and SCN9A, and 22% expressed SCN10A which is consistent with the patch clamp data (presented elsewhere). **(B)** uMAP plots showing the majority of cells cluster as mature sensory neurons (~91%) and with the remaining cells classifying as sensory progenitor (~9%). Individual uMAP plots of non-opioid pain targets show they are expressed within the mature sensory neuron population.

Robust Calcium Response with Enhanced Calcium Pathway Gene Expression





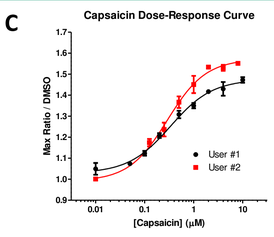


Figure 5. Robust Calcium Responses to Sensory Receptor Agonists. (A) RNAseq pathway and Go analysis comparing iCell Sensory Neurons vs. all other protocols identified significantly up- or down-regulated pathways. Importantly, calcium handling pathways were significantly enriched in iCell Sensory Neuron, which is beneficial for sensory agonist calcium influx assays. **(B)** Representative traces of calcium response to a titration of Capsaicin (0→8 µM, TRPV1 agonist) from the FDSS/µCell. **(C)** Assay reproducibility demonstrated by two independent operators generating similar results from a dose-response of Capsaicin with the same lot of iCell Sensory Neurons. **(D)** Compound profiling in 384-well format: six different TRPV1 antagonists (dose-response, n=3) were incubated for 1 h prior to stimulation with 1 µM Capsaicin. EC_{50} values are sub-micromolar.

Expression and Function of Migraine-Related Pain Targets

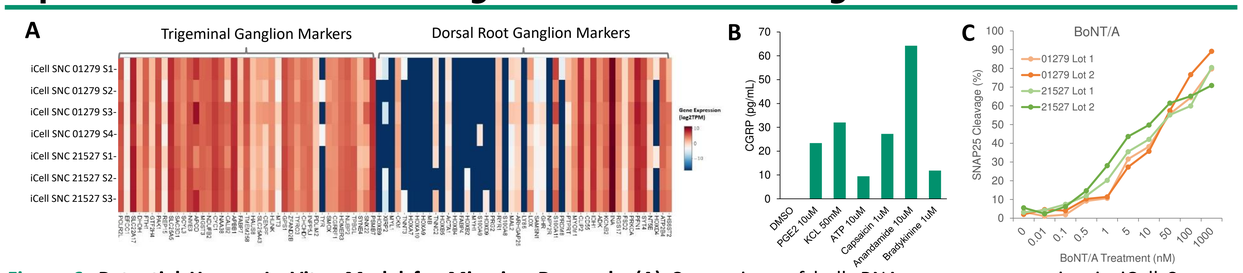


Figure 6. Potential Human In Vitro Model for Migraine Research. (A) Comparison of bulk RNAseq gene expression in iCell Sensory Neurons across identified trigeminal and dorsal root ganglion genes (*Megat et al., J. of Nsci., 2019*), suggesting a human trigeminal neuron genotype. **(B)** iCell Sensory Neurons released CGRP when stimulated by various pain modulators as measured by ELISA (KCl as positive control). **(C)** Quantitative dose-dependent SNAP25 cleavage upon exposure to a broad range of Botulinum Toxin A (BoNT/A) detected by Western blot.

Conclusions

FUJIFILM CDI has developed a robust differentiation protocol for large-scale manufacturing of human iPSC-derived sensory neurons.

iCell Sensory Neurons:

- Have a distinct bulk RNASeq profile compared to hDRG, but express many non-opioid pain-relevant genes, including Nav1.7, Nav1.8 and TRPV1. Some genes are enhanced compared to sensory neurons derived using alternate differentiation protocols.
- Demonstrate enhanced calcium pathway gene expression and robust calcium influx responses to sensory agonists, indicating their utility for high throughput drug screening.
- Release CGRP when stimulated, show SNAP25 cleavage in response to BoNT/A, and expression of trigeminal neuron markers, demonstrating usage as a model system for migraine study.

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