Pain in a Dish

Clifford J Woolf
Pharma Return on Investment

Overall trend in R&D efficiency (inflation-adjusted)

- FDA tightens regulation post-thalidomide
- FDA clears backlog following PDUFA regulations plus small bolus of HIV drugs
- First wave of biotechnology-derived therapies

Scannell et al Nature reviews Drug Discovery 2012

Decline in approved drugs per billion US$ spent on R&D
Drug Target High Throughput Screening

What Target?
Heterologous expression in HEK or CHO cells does not replicate native receptor properties
Study human receptors in human neurons
Phenotypic screen - disease in a dish
Directed human neuronal differentiation

Embryonic/IP Stem cells → Differentiation factors → Specific neuron population
Disease Modeling

New Therapy

Disease Model

Skin Punch Biopsy

Skin Fibroblasts

Direct Reprogramming

In Vitro Differentiation

Patient Specific Stem Cells

Neurons and Astrocytes
Neuronal specification in patient iPSC lines

DNA  ISL  TUJ1

SOD1A4V 39b

Shinya Yamanaka  Kevin Eggan
What about pain?
Peripheral Terminal

Transducer Channels
TRPV1-4
TRPM8
TRPA1
ASICs
P₂X₃
TREK
TASK

Voltage-Gated Channels
1.7, 1.8 & 1.9

Noxious Stimulus

Transduction

Nav1.8 – tomato
Screen for analgesic efficacy in human nociceptors
Study pain channelopathies in human neurons
Determine risk of developing pain/neuropathy
Directed differentiation

**Undifferentiated hESC**

- **Day 0**: Make EB
  - hEB media: 10%KSR, 10% FBS, GlutaMax, NEAA, Pen/Strep, β-ME

**EB**

- **Day 11**: Treat with 100nM RA
  - N2 media: DMEM/F12, GlutaMax, N2 Supplement, Pen/Strep, D-Glucose, Ascorbic Acid

**Neural progenitors**

- **Day 14**: Dorsalize
  - Treat with 20ng/ml BMP-4 and 1µM Cyclopaamine

**Neuron**

- **Day 28**: Dissociation with Papain
  - Plate the cells onto polyornithine and fibronectin (2.5µg/ml) coated plate
  - Nociceptor medium: Neurobasal, B27, N2, GlutaMax, Pen/Strep, D-Glucose, Ascorbic Acid, 25ng/ml NGF, 20ng/ml BDNF, 20ng/ml GDNF (2% FBS for first 2 days)

- **Day X**: Fix cells
  - Perform immunostaining and evaluate the expression profile of marker genes
  - Antibodies: Brn3a, Peripherin, Runx1, TrkA, Ret, Islet1, TRPV1

**HUES lines**

- HUES1
- HUES2
- HUES3
- HUES4
- HUES5
- HUES6
- HUES7
- HUES8
- HUES9
- HUES10
- HUES11
- HUES12
- HUES 13
- HUES14
- HUES15
- HUES16
- HUES17
Transdifferentiation

Isolate MEFs from NaV1.8::tomato mice.

Transduce MEFs with BAM and nociceceptor factors.

Detect neuronal morphology and activation of tomato reporter.
Voltage clamp

Phenotyping Nociceptors

Calcium Imaging
Which Transcription factors?

Neural crest

- HNK-1
- p75
- Sox10
- FoxD3
- Pax3
- Slug
- Snail

Neurogenesis
E10.5-E12

- Ngn1

Early differentiation
E11-E13

- Brn3a
- Islet1
- Tlx3
- DRG11

- Postmitotic nociceptor

Maturation
E15-P14

- TrkA
- Runx1

Immature nociceptor

Mature Nociceptor

- Runx1
- Ret

Non-peptidergic

- TrkA

Peptidergic
Transcriptional profiling at whole population and single cell levels reveals somatosensory neuron molecular diversity

Isaac M Chiu¹,²,⁶*, Lee B Barrett¹,², Erika K Williams³, David E Strochlic³, Seungkyu Lee¹,², Andy D Weyer⁴, Shan Lou⁵, Gregory Bryman¹,², David P Roberson¹,², Nader Ghasemlou¹,², Cara Piccoli¹,², Ezgi Ahat¹,², Victor Wang¹,², Enrique J Cobos¹,²,⁷, Cheryl L Stucky⁴, Qiufu Ma⁵, Stephen D Liberles³, Clifford J Woolf¹,²*
Three years .....................
Five factors & four weeks…

- Plate Split Plat-E Cells
- Add virus to MEF culture
- Media & Growth Factors

D 1-3 D 5 Fibroblasts D 7 D 9 D 10 Neurons

Co-culture with Glia
A combination of 5 factors results in efficient production of tomato-positive neurons.
### What are the 5 factors?

<table>
<thead>
<tr>
<th>Gene</th>
<th>Source</th>
<th>Family</th>
<th>Role in Reprogramming/Sensory System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascl1 (Achaete-Scute complex homolog1)</td>
<td>Lit</td>
<td>Basic helix-loop-helix/achaete-scute</td>
<td>Neuronal lineage reprogramming/neuronal commitment</td>
</tr>
<tr>
<td>Isl2 (Insulin gene enhancer protein2)</td>
<td>Exp, BioGPS</td>
<td>Homeo-domain/LIM region</td>
<td>Unknown *</td>
</tr>
<tr>
<td>Klf7 (Kruppel-like factor 7)</td>
<td>Lit, Exp, BioGPS</td>
<td>Zinc-finger/Krueppel like</td>
<td>TrkA maintenance</td>
</tr>
<tr>
<td>Myt1l (myelin TS factor1)</td>
<td>Lit</td>
<td>Zinc-finger</td>
<td>Neuronal lineage reprogramming</td>
</tr>
<tr>
<td>Ngn1 (Neurogenin1)</td>
<td>Lit</td>
<td>Basic helix-loop-helix</td>
<td>TrkA and subsequent TrpV1 expression</td>
</tr>
</tbody>
</table>

* motor neuron identity, development and placement

Allen Brain Atlas
Expression of Nociceptor-Specific mRNAs

Fold difference in mRNA level relative to MEFs

- S100A4
- SCN9A
- NTRK1

- DRGs
- iNoc
Molecular characterization of induced neurons
Electrophysiology of Induced Nociceptors
Nav1.7 current in miNoci $\rightarrow$ ProTxII

$V_{1/2}$ ACT miNoci
$Nav1.7 = -23$ mV

$V_{1/2}$ DRG
$Nav1.7 = -31$ mV

$V_{1/2}$ INA miNoci
$Nav1.7 = -71$ mV

$V_{1/2}$ DRG
$Nav1.7 = -76$ mV
Action Potential and Firing Properties

- **iNoc**
  - 20mV
  - 5ms

- **Primary Noc**
  - 20mV
  - 100ms

- **Primary Non-Noc**
Induced Nociceptors Fire in Response to Trp Agonists

- capsaicin
- mustard oil

+ capsaicin
+ mustard oil

10 uV
0.5 s
Sensitization by Inflammatory Mediators

![Graph showing the response to capsain in the presence of inflammatory mediators like PGE2 and KCl. The graph depicts the change in fluorescence (ΔF/F) over time (30 s). The response is plotted against time after initial exposure to capsain.](image-url)
Transmitter release

CGRP (pg/ml)

iNoc  BAM  iNoc  BAM
KCl  Vehicle
Total Firing Frequency Over Recording Session

Instantaneous Spike Frequency [spike/second]

Time (s)

1 μm capsaicin
Induced Human neurons
Induced neurons from a patient with FD differ from healthy control induced neurons.
Induced neurons can be used to screen for neurotoxic effects of cancer chemotherapeutic agents

oxaliplatin
Modeling pain *in vitro* using nociceptor neurons reprogrammed from fibroblasts

Brian J Wainger¹⁻³,⁸, Elizabeth D Buttermore¹,³,⁸, Julia T Oliveira¹,⁴, Cassidy Mellin¹, Seungkyu Lee¹,³, Wardiya Afshar Saber¹, Amy J Wang¹, Justin K Ichida⁵,⁶, Isaac M Chiu¹,³, Lee Barrett¹, Eric A Huebner¹,³, Canan Bilgin¹, Naomi Tsujimoto⁵, Christian Brenneis¹, Kush Kapur¹, Lee L Rubin⁵, Kevin Eggin⁵,⁷ & Clifford J Woolf¹,³
A

sgRNA a

|------------------| +n bp sgRNA offset |

|------------------| 5' overhang        |

5' --------- target b --- 3'

3' --------- target a --- 5'

sgRNA b

---

Cas9

DSB

5' = = = = = = = = 3'

NHEJ

sgRNA

HDR

---

5' = = = = = = = = 3'

Indel mutation

Premature stop codon

5' = = = = = = = = 3'

Genomic 5'

DNA 3'

Repair 5'

template 3'

Precise gene editing

5' = = = = = = = = 3'

3' = = = = = = = = 5'
**Na\textsubscript{v} 1.7 mutations**

- **Loss of function** → Insensitivity to pain (CIP)
- **Gain of function** → Chronic pain syndromes
- Erythromelalgia (IEM)
- Paroxysmal extreme pain disorder (PEPD)
- Idiopathic small fiber neuropathy (SFN)

**Diagram Details:**
- **DI**
  - VSD
  - PM
- **DII**
  - S1
  - S2
  - S3
  - S4
  - S5
  - S6
- **DIII**
  - L1
  - L2
- **DIV**
  - L3

**Legend:**
- IEM (F1449V)
- PEPD
- SFN
Brian Wainger  Liz Buttermore

Justin Ichida  Wardiya Afshar  Isaac Chiu

Lee Rubin  Kevin Eggan

Cedric Laedermann  Inge Chen  Lendy Chu