OPEN

Investigating Peripheral Regional Anesthesia Using Induced Pluripotent Stem Cell Technology: Exploring Novel Terrain

Pascal S. H. Smulders, MD,* Mark L. van Zuylen, MD,* Jeroen Hermanides, MD, PhD,* Markus W. Hollmann, MD, PhD,* Werner ten Hoope, MD,*† and Nina C. Weber, PhD*

GLOSSARY

3i = 3 small-molecule inhibitors; **DRG** = dorsal root ganglion; **ESC** = embryonic stem cell; **iPSC** = induced pluripotent stem cell; **LSB** = LDN-193189 and SB431542; **MEA** = microelectrode array; **Na$_v$** = voltage-gated sodium channel; **NCC** = neural crest cell; **RA** = peripheral regional anesthesia; **TRPV1** = transient receptor potential vanilloid 1; **TTX** = tetrodotoxin

In vitro research on peripheral regional anesthesia (RA) has historically been performed using animal cell types due to the challenges of obtaining human adult or fetal dorsal root ganglion (DRG) neurons. However, translation of results from animal studies is often problematic because of genetic differences between animals and humans. The discovery of human induced pluripotent stem cells (iPSCs) now offers an opportunity to circumvent the obstacles of acquiring human neuronal tissue, as it allows conventional human cells to be reprogrammed to nociceptive DRG neurons. Here, we explain iPSC technology and focus on how it could facilitate drug research and personalized medicine for RA.

From the *Laboratory of Experimental Intensive Care and Anesthesiology (L.E.I.C.A.), Department of Anesthesiology, Amsterdam UMC Location AMC, Amsterdam, the Netherlands and †Department of Anesthesiology, Rijnstate Hospital, Arnhem, the Netherlands.

Accepted for publication February 25, 2022.

Funding: Funding was provided by the Amsterdams Universiteitsfonds.

The authors declare no conflicts of interest.

Reprints will not be available from the authors.

W. ten Hoope and N. C. Weber contributed equally.

Address correspondence to Markus W. Hollmann, MD, PhD, Department of Anesthesiology, Amsterdam UMC location AMC, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands. Address e-mail to m.w.hollmann@amsterdamumc.nl.

Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the International Anesthesia Research Society.

This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

DOI: 10.1213/ANE.0000000000006018

PROGRESS IN INDUCED PLURIPOTENT STEM CELL TECHNOLOGY

The narrative of iPSC technology starts with landmark experiments in which tadpoles were generated from terminally differentiated intestinal epithelial nuclei. These studies demonstrated that specialized cells retain a full retinue of genes and inspired the concept of reprogramming.[1] The development of preimplantation embryo-derived cell lines, now known as embryonic stem cells (ESCs), then led to cell fusion experiments that suggested the existence of transcription factor genes involved in reprogramming.[2] These reports ultimately culminated in the seminal discovery of iPSCs.[3] This man-made stem cell class possesses similar differentiation potential as ESCs, but is not burdened by the ethical and regulatory issues that make handling of ESCs impractical.[4]

First described in 2006, iPSC production involves the forced introduction of transcription factors into a somatic host cell.[3] The somatic cell subsequently regresses to a pluripotent state, reacquiring the ability to differentiate into any cell type of the body (pluripotency) and to divide indefinitely, forming unaltered daughter cells (self-renewal) (Figure 1). The obtained iPSCs can then be differentiated into the cell type of interest using growth factors and small molecules. Neural cell types can thus be generated from easily obtainable cells, such as skin fibroblasts and umbilical cord blood cells, and expanded unlimitedly, placing previously unattainable human cell models within reach. Recent breakthroughs in stem cell biology further improved the quality of iPSC technology and paved the way for its utilization in disease modeling and drug discovery in nonspecialized (anesthesiology) laboratories.[4,6]

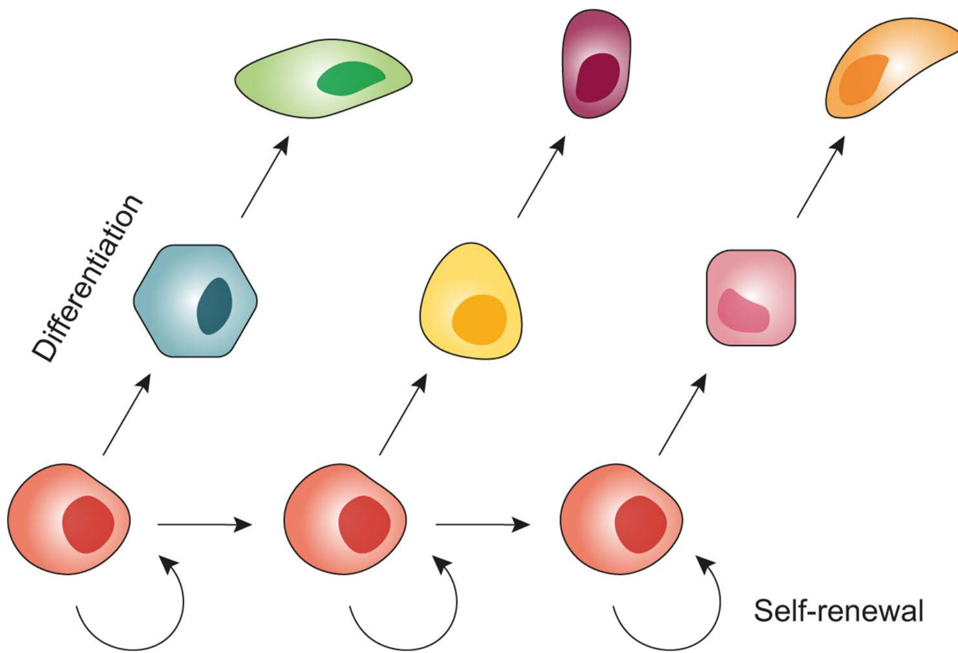

Figure 1. iPSCs are capable of differentiation into cell types from all 3 germ layers of the human body (pluripotency; depicted on the vertical axis by the different cellular shapes and colors), and indefinite division that creates unaltered daughter cells (self-renewal; illustrated on the horizontal axis by a circular arrow for the first [identical] daughter cell, and a straight arrow for the formation of a second [identical] daughter cell). Inspired by Kolios and Moodley.[5] iPSC indicates induced pluripotent stem cell.

DIFFERENTIATING DORSAL ROOT GANGLION NEURONS FROM INDUCED PLURIPOTENT STEM CELLS

Neural induction is the first step in the development of the nervous system. Blockade of Smad phosphorylation (among other pathways) prevents the differentiation of ectodermal cells toward an epidermal fate, resulting in the columnar neuroepithelial cells that form the neural plate. Subsequent elongation, folding, and closing of the neural plate form the neural tube during neurulation. The neural tube then evolves into the brain and spinal cord, while the peripheral nervous system differentiates (except for neurons innervating the face) from neural crest cells (NCCs) that delaminate from the dorsal section of the rudimentary neural tube. Next, part of the truncal NCCs aggregate into segmental clusters lining the spinal cord which, after terminal differentiation, form the DRG. Interestingly, the most common approach to nociceptive DRG neuron conversion leads iPSCs through stages strongly resembling the aforementioned embryological steps.

The substantial contributions made by Chambers et al[7,8] form the foundation of lineage-based DRG nociceptor reprogramming (Figure 2). The process starts with the induction of pluripotency in the host cell through transient ectopic expression of reprogramming factors, which is continued until endogenous genes maintain pluripotency. Neural differentiation is later started by application of drugs inhibiting Smad signaling, resulting in colonies of columnar epithelial cells (or neural rosettes) that resemble the developing neural tube.[8,9] Subsequent parallel treatment with 3 small molecule

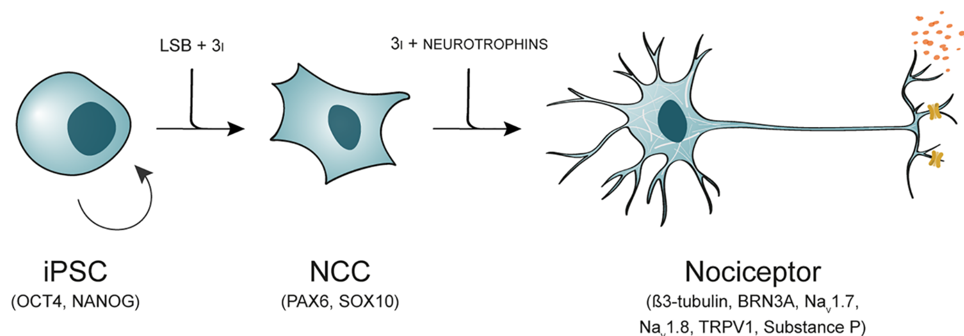

Figure 2. Lineage-based reprogramming follows steps comparable to embryological development. Conversion starts with an iPSC that is pluripotent and self-renewable in nature. Subsequent Smad-inhibition (depicted using the abbreviation LSB for the compounds LDN-193189 and SB431542) and application of 3i directs differentiation toward an NCC identity. Parallel treatment with a mixture of 3i and neurotrophins settles the DRG nociceptor fate. Correct differentiation can be determined using standard laboratory techniques, as differentiating cells possess markers corresponding to their (current) state. Mature nociceptive neurons, for example, will express neural cytoskeleton marker β3-tubulin, voltage-gated sodium channels (eg, Na_v 1.7) and transient receptor potential channels (eg, TRPV1), and excrete substance P, as shown here. 3i indicates 3 small-molecule inhibitors; DRG, dorsal root ganglion; iPSC, induced pluripotent stem cell; LSB, LDN-193189 and SB431542; Na_v 1.7/8, voltage-gated sodium channel isoform 1.7/8; NCC, neural crest cell; TRPV1, transient receptor potential vanilloid 1.

inhibitors then settles differentiation from an NCC subphase to a DRG nociceptor fate.[7] The obtained early nociceptors are then matured using a cocktail of neurotrophins to acquire functionally mature nociceptive neurons that secrete the neurotransmitter substance P, which (in vivo) transmits information to second-order neurons.[7] Similar to in vivo DRG neurons, iPSC-derived nociceptors are electrically active and coalesce into ganglion-like structures.

Central to successful iPSC modeling is correct differentiation and stepwise quality control. DRG nociceptors lose and gain expression of genes corresponding to the different developmental stages during differentiation. For example, iPSCs will lose expression of pluripotency markers (eg, OCT4 and NANOG), while transiently expressing neuroectoderm PAX6 and NCC marker SOX10. Cells then gain a nociceptive neuron profile coexpressing neural β3-tubulin, sensory neuron-specific BRN3A, and nociceptive markers, such as voltage-gated sodium channels (Na_v) and transient receptor potential channels. Transition through these formative steps can be confirmed with immunofluorescence imaging applications that detect the aforementioned antigens using fluorescent-labeled antibodies. In addition, optical microscopy can visualize the morphological changes that occur as the initially rounded iPSCs elongate, grow neurites, and group into ganglion-like clusters during nociceptor conversion.

Electrophysiological assays are considered the gold standard for functional analysis of iPSC-derived nociceptors. Microelectrode array (MEA) enables extracellular recording of action potentials by coupling neuronal activity to electronic circuitry in a noninvasive manner. Therefore, MEA can affirm nociceptive neuron identity by detailing electrical activity of neuronal networks cultured over electrodes embedded within the culture plate. For example, treatment with capsaicin, an agonist of transient receptor potential vanilloid 1 (TRPV1), would trigger noxious signaling in the form of ionic current changes measurable as action potentials, while application of tetrodotoxin (TTX) could attest to the presence of TTX-reactive Na_vs. Whereas MEA enables high-throughput screening by extracellular measurements, patch clamping provides detailed intracellular recordings of an individual cell. In patch clamping, a high resistance seal is formed between the cell membrane and a micropipette, giving the experimenter access to the interior of the cell. Therefore, patch clamping allows for mechanistic studies that evaluate ionic currents and membrane channel properties, thus complementing MEA.

OPPORTUNITIES FOR PERIPHERAL REGIONAL ANESTHESIA

RA has evolved significantly over the last decades and has become an important component of balanced anesthesia.[10] Yet, the field could benefit from a research model that better reflects the human neurobiological intricacies of pain signaling than the currently available animal models.

Pain-related research results obtained from animal studies are often difficult to translate to the human situation due to heritable neural interspecies differences. For example, the expression patterns and electrophysiological properties of TRPV1 and Na_v 1.7 and 1.8, canonical markers of pain signaling, differ significantly between humans and rodents.[11,12] Genetic differences could, thus, help explain the faltering discovery of novel anesthetic medication and nerve block strategies, supporting the need of a human cell model of RA. As described above, the progress in iPSC technology now allows reliable differentiation of human DRG neurons for use in preclinical research. Human iPSC-derived RA models could, thus, accommodate progressive drug testing and personalized medicine.

iPSC technology holds great potential for pharmaceutical research as it provides a noninvasive method of obtaining scalable quantities of (difficult to access) human cells that can be used for both functional analysis and (neuro)toxicity testing. As such, one of the main applications of the technique coincides with a major goal of RA: to find a selective long-lasting anesthetic agent that outlasts surgical pain and has minimal adverse effects.[13,14] To date, no such drug has been found, leading clinicians to seek an alternative in additives (eg, α_2 agonists and dexamethasone) to local anesthetics. Although some of these combinations appear to result in prolongation of nerve blockade, additives fail to deliver the steerable and lasting effect that is sought for. Furthermore, the effect size and mechanism of action are often unknown, while data on neurotoxicity are unavailable due to off-label usage. Within this context, a human iPSC-derived RA model would enable the testing of novel drugs without the interference of other cell types or organ systems. Researchers could first screen for the presence of a nociceptor-mediated effect of a compound using MEA and subsequently perform patch-clamping measurements to determine the exact result on ionic membrane currents. Toxicity assays would then complete a comprehensive preclinical screening of a potential analgesic. Similar methods could be used to investigate the properties of established additives and other relevant drugs. When adapted, the described system would also offer chances for research into treatment of chronic pain and painful neuropathies (eg, by inducing a neuropathic phenotype through the application of chemotherapeutics). Although iPSC technology is increasingly used for drug discovery in other specialties, it remains underutilized in anesthesiology and pain medicine.

The reprogramming process preserves the genetic code of the original somatic cell, including all

(disease-related) mutations and variations. Therefore, single-donor iPSC lines are representative of the donor and offer the opportunity to perform experiments on patient-specific cells that were previously unavailable. iPSC technology, thus, enables a more personalized approach to RA (research); iPSCs can provide a "trial in a dish" for underrepresented patient categories, complementing large clinical studies. RA-relevant genetic mutations (eg, channelopathies) and diseases (eg, neuropathies) can be reproduced and studied to provide directly translatable results, where this was previously impossible or impractical. In addition, a patient with a previously unexplained complication of RA, or a positive family history of such, could donate cells for reprogramming that may reveal an altered response to medication relevant to a planned surgical procedure.

iPSC reprogramming is a still evolving technique that has limitations. Currently, reprogramming efficiency is still relatively low, making iPSC culture expensive compared to other cell culture systems.[15] Contributing to the costs are the long culture time required for terminal differentiation and the associated labor-intensive nature of the sensitive cells.[16] Another factor to consider is the maturation status of the differentiated cells. Although the converted cells are morphologically and functionally similar to their in vivo counterparts, the differentiated cells might not be epigenetically identical.[17,18] New protocols and methods are constantly being developed, and it is expected that these issues will be resolved in the coming years.

Although new to anesthesiology, human iPSCs provide a translatable platform that is directly applicable to RA. iPSC technology, thus, provides a method for performing progressive human disease modeling and drug discovery. As such, it is an opportunity to further elevate the status of RA and could contribute to the future of anesthesiology as a perioperative specialty.

ACKNOWLEDGMENTS

The authors thank Simone Kersten, MSc, for creating the figures for this article.

DISCLOSURES

Name: Pascal S. H. Smulders, MD.
Contribution: This author helped conceive and write the manuscript.
Name: Mark L. van Zuylen, MD.
Contribution: This author helped conceive and write the manuscript.
Name: Jeroen Hermanides, MD, PhD.
Contribution: This author helped conceive and write the manuscript.
Name: Markus W. Hollmann, MD, PhD.
Contribution: This author helped conceive and write the manuscript.
Name: Werner ten Hoope, MD.
Contribution: This author helped conceive and write the manuscript.
Name: Nina C. Weber, PhD.
Contribution: This author helped conceive and write the manuscript.
This manuscript was handled by: Alexander Zarbock, MD.

REFERENCES

1. Gurdon JB. The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *J Embryol Exp Morphol.* 1962;10:622–640.
2. Tada M, Takahama Y, Abe K, Nakatsuji N, Tada T. Nuclear reprogramming of somatic cells by in vitro hybridization with ES cells. *Curr Biol.* 2001;11:1553–1558.
3. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell.* 2006;126:663–676.
4. Omole AE, Fakoya AOJ. Ten years of progress and promise of induced pluripotent stem cells: historical origins, characteristics, mechanisms, limitations, and potential applications. *PeerJ.* 2018;6:e4370.
5. Kolios G, Moodley Y. Introduction to stem cells and regenerative medicine. *Respiration.* 2013;85:3–10.
6. Takahashi K, Yamanaka S. A decade of transcription factor-mediated reprogramming to pluripotency. *Nat Rev Mol Cell Biol.* 2016;17:183–193.
7. Chambers SM, Qi Y, Mica Y, et al. Combined small-molecule inhibition accelerates developmental timing and converts human pluripotent stem cells into nociceptors. *Nat Biotechnol.* 2012;30:715–720.
8. Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol.* 2009;27:275–280.
9. Wilson PG, Stice SS. Development and differentiation of neural rosettes derived from human embryonic stem cells. *Stem Cell Rev.* 2006;2:67–77.
10. Mulroy M. A History of Regional Anesthesia. In: Eger Ii EI, Saidman LJ, Westhorpe RN, eds. *The Wondrous Story of Anesthesia.* Springer, NY; 2014:859–870.
11. Rostock C, Schrenk-Siemens K, Pohle J, Siemens J. Human vs. mouse nociceptors - similarities and differences. *Neuroscience.* 2018;387:13–27.
12. Han C, Estacion M, Huang J, et al. Human Na(v)1.8: enhanced persistent and ramp currents contribute to distinct firing properties of human DRG neurons. *J Neurophysiol.* 2015;113:3172–3185.
13. Jablonski S, Lirk P. Future in regional anesthesia and pain medicine: the pharmacological view. *Minerva Anestesiol.* 2021;87:351–357.
14. Boezaart AP, Zasimovich Y, Parvataneni HK. Long-acting local anesthetic agents and additives: snake oil, voodoo, or the real deal? *Pain Med.* 2015;16:13–17.
15. Malik N, Rao MS. A review of the methods for human iPSC derivation. *Methods Mol Biol.* 2013;997:23–33.
16. Lampert A, Bennett DL, McDermott LA, et al. Human sensory neurons derived from pluripotent stem cells for disease modelling and personalized medicine. *Neurobiol Pain.* 2020;8:100055.
17. Bilic J, Izpisua Belmonte JC. Concise review: induced pluripotent stem cells versus embryonic stem cells: close enough or yet too far apart? *Stem Cells.* 2012;30:33–41.
18. Karagiannis P, Takahashi K, Saito M, et al. Induced pluripotent stem cells and their use in human models of disease and development. *Physiol Rev.* 2019;99:79–114.