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Cells in Focus

Modelling the dorsal root ganglia using human pluripotent stem cells: A platform to study peripheral neuropathies

Serena Viventi[a], Mirella Dottori[a,b,*]

[a] Department of Biomedical Engineering, University of Melbourne, Australia
[b] Illawarra Health and Medical Research Institute, Centre for Molecular and Medical Bioscience, University of Wollongong, Australia

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ABSTRACT

Sensory neurons of the dorsal root ganglia (DRG) are the primary responders to stimuli inducing feelings of touch, pain, temperature, vibration, pressure and muscle tension. They consist of multiple subpopulations based on their morphology, molecular and functional properties. Our understanding of DRG sensory neurons has been predominantly driven by rodent studies and using transformed cell lines, whereas less is known about human sensory DRG neurons simply because of limited availability of human tissue. Although these previous studies have been fundamental for our understanding of the sensory system, it is imperative to profile human DRG subpopulations as it is becoming evident that human sensory neurons do not share the identical molecular and functional properties found in other species. Furthermore, there are wide range of diseases and disorders that directly/indirectly cause sensory neuronal degeneration or dysfunctionality. Having an *in vitro* source of human DRG sensory neurons is paramount for studying their development, unique neuronal properties and for accelerating regenerative therapies to treat sensory neuropathies. Here we review the major studies describing generation of DRG sensory neurons from human pluripotent stem cells and fibroblasts and the gaps that need to be addressed for using *in vitro*-generated human DRG neurons to model human DRG tissue.

1. Introduction

The fundamental purpose of the sensory nervous system is to receive and transmit information from skin, muscle and sensory organs to the brain, which initiates how we interpret our external and internal world and consequently influences what response will be made. The sensory nervous system includes the visual, auditory and olfactory systems as well as the peripherally located cranial sensory and dorsal root ganglia (DRG). The DRG consists of a multitude of sensory neuronal subtypes that function to relay sensory stimuli, including temperature, pressure, pain and position to the central nervous system.

Not surprisingly, there are vast ranges of diseases and conditions (Melli and Höke, 2009), usually progressive, which can affect DRG sensory neurons. The underlying causes of DRG degeneration may be either directly intrinsic to DRG neurons or indirectly associated with other pathologies. Some inherited genetic diseases inducing DRG degeneration include Friedreich's Ataxia (Delatycki and Corben et al., 2012) and Charcot Marie Tooth Disease (d'Ydewalle et al., 2012). Other conditions that may affect DRG neuronal functionality are autoimmune disease (Sederholm, 2010), infections (such as AIDS) (Schütz and Robinson-Papp et al., 2013) or unknown idiopathic conditions (de Schryver et al., 2011). DRG degeneration is also observed in cases relating to drugs (Manji, 2011) and alcohol toxicity (Chopra and Tiwari, 2012). DRG neurons can also be severely damaged by physical trauma (Lim et al., 2015). People affected by DRG peripheral neuropathy report different symptoms, including mild to chronic pain, loss of feeling and intense itchiness. Such symptoms can be severely debilitating depending on the nature and cause. Often the degeneration is progressive and, for some conditions, treatments are geared to damping the symptoms and slowing progression of nerve damage. In many cases, there is no treatment available. Having an *in vitro* source of human sensory neurons would be invaluable for developing neuroregenerative therapies to treat specific neuropathies.

1.1. Classification of DRG sensory neurons

Somatosensory neurons of the DRG are traditionally classified into three sub-populations: nociceptors, mechanoreceptors and proprioceptors. This classification is based on characteristics relating to their function and morphological features such as cell body size, axon diameter, degree of myelination, types of afferent endings and the laminar targets of their efferent terminals in the spinal cord (Kandel et al.,

* Corresponding author at: Illawarra Health and Medical Research Institute, Building 32, University of Wollongong, NSW, 2522, Australia.
E-mail address: mdottori@uow.edu.au (M. Dottori).

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2013). However, studies conducted on transgenic mice and more sophisticated molecular techniques have provided a breakthrough in this field, revealing a more complex and subtle diversity among these three subpopulations. Emerging new knowledge enables the possibility to improve the traditional classification and adopt new criteria to classify the somatosensory neurons based on in-depth transcriptional profiling matched with functional analyses. A general overview of the three major classes of DRG sensory neurons are:

Nociceptive neurons respond to painful or pruritic (itch) stimuli and thermoception (Dubin and Patapoutian, 2010; Zhang and Bao, 2006). They innervate peripheral tissues such as cutaneous epithelial tissue, as well as muscle and other internal organs, where molecular receptors located on sensory terminals react to noxious stimuli (Proske and Gandevia, 2012). They are classified as small diameter (< 30 μm) and can be distinguished several subtypes depending on the rate of myelination: unmyelinated (C) or lightly myelinated (Aδ) fibres. Unmyelinated C fibre neurons are characterized by slow conductivity (~2 m/s) and are able of responding to different combination of stimuli, including temperatures, pruritogens, tissue damage, chemical irritants (Laing and Dhaka, 2016). Myelinated Aδ fibre neurons have faster conduction (up to 30 m/s) and, as consequence of this property, are specialized for detecting localized fast pain, repeated stimulation as well as tissue injury (Laing and Dhaka, 2016). The neurotrophic receptor expressed by nociceptors is TrkA, that has high affinity for Nerve Growth Factor (NGF). There is a smaller population of nociceptive neurons that transiently express TrkA during development, and eventually express Ret (Luo et al., 2007). Nociceptors are predominantly excitatory neurons and release glutamate as their primary neurotransmitter. They can be further sub-classified into peptidergic and non-peptidergic neurons. Peptidergic nociceptive neurons release neuropeptides such as substance P or calcitonin gene related-peptide (CGRP), instead non-peptidergic neurons are identified by the expression of isolectin IB4 (a histological marker) (Mandge and Manchanda, 2015).

Proprioceptive neurons transmit information about conscious sensations, such as limb position, balance and movement. They consist of large (12–20 μm) and medium (6–12 μm) diameter myelinated A fibres (Aα, Aβ). Proprioceptors express TrkC neurotrophic receptors, activated by Neurotrophin 3 (NT-3) (Fariñas et al., 1998; Henion et al., 1995; Liebl et al., 1997; Tessarollo et al., 1997). Due to their degree of myelination and diameter size, they have a fast conduction velocity (72–120 m/s for large proprioceptors and 30–70 m/s for medium ones) (Le Pichon and Chesler et al., 2014). Proprioceptors innervate specialised organs called Golgi tendon organs and muscle spindle. They function to sense muscle tension, contraction and limb position. Their efferent projections mostly end in laminae III-V of the spinal cord. However, some also make connections in deeper laminae in order to communicate with motor neurons of reflex circuits (Le Pichon and Chesler et al., 2014).

Mechanoreceptors innervate skin tissue and detect cutaneous touch sensation and vibrational stimuli. Most mechanoreceptor fibres are large diameter (Aβ) (Abraira and Ginty, 2013; Le Pichon and Chesler et al., 2014) myelinated and usually distinguished by their expression of TrkB receptor which has high affinity for Brain-derived growth factor (BDNF) (Shimizu et al., 2007). Mechanoreceptors project to the dermal and epidermal regions of the skin and function to detect mechanical stimuli *via* specialised mechanosensory end organs such as Meissner's corpuscles, Pacinian corpuscles, Ruffini corpuscules and Merkel cell endings (Fleming and Luo, 2013; Gilman, 2002; Zimmerman et al., 2014).

These classification systems have served well but with the advent of better molecular techniques we know that they may not adequately cover the true diversity or the lineage origin. New emerging studies using single cell gene expression profiling of adult rodent DRGs have identified novel subpopulations, as well as re-defined gene expression profiles of more commonly known populations (Li et al., 2017, 2016; Usoskin et al., 2015). To date most of the molecular and functional profiling of sensory DRG subclasses has been driven by animal studies and less is known about human DRG neurons. The few studies describing human DRG sensory neurons show inconsistencies in molecular and functional properties compared to that described in the rodent. Some examples include: P2 × 3 receptors that are involved in mediating chronic pain show different potencies in their response to antagonists between rodent and primate DRG neurons (Serrano et al., 2012); pharmacological agents that effectively block $GABA_A$ receptor currents in rodent DRG neurons are not effective in human DRGs (Valeyev et al., 1996); the family of mas-related G-protein-coupled receptors (MRGPRS) associated with nociceptor neurons consist of 4 genes in the human genome but over 30 genes within the mouse genome (Zylka et al., 2003); there are differences in the proportion of DRG neurons that co-express TRPV1/TRKA and RET/TRKA between adult human and mouse DRG tissue (Rostock et al., 2017); NF200 expression is detected in most human DRG neurons but only in a subset of mouse DRGs (Rostock et al., 2017). These studies and others strongly highlight the need to work with human sensory neurons, particularly for screening candidate pharmaceutical compounds that modulate adverse sensations, such as chronic pain.

2. Human sources of dorsal root ganglia sensory neurons

There is a strong need to obtain an *in vitro* source of human sensory neurons to study their unique molecular and functional profiles, myelination properties, regenerative capacities and, in particular, for establishing high through-put drug screening platforms. Whilst there are reports describing human sensory neurons derived from human DRG tissue (Davidson et al., 2014; Rostock et al., 2017; Valtcheva et al., 2016; Zhang et al., 2017), this source is impractical for many laboratories. With the discovery of human pluripotent stem cells (hPSC), it is now feasible to derive human sensory neurons in abundance and minimize the need to access human DRG tissue for advancing therapies and knowledge about the human sensory system. The review below details some of the major studies describing generation of human DRG neurons from hPSC, outlining the approaches used, characterization of the neurons and their applications. An alternative approach, whereby sensory neurons are generated directly from human fibroblasts, is also described.

2.1. Human pluripotent stem cells

Pluripotent stem cells are stem cells that have the ability to differentiate to all cell types of body. There are two different avenue by which human pluripotent stem cells (hPSC) can be generated; (a) from embryos (human embryonic stem cells, hESC), and (b) *via* genetic reprogramming of somatic cells to induce pluripotent stem cells (iPSC) (Hirschi et al., 2014; Takahashi and Yamanaka, 2006). IPSC technologies have allowed the possibility of modifying patient-derived cells to regain their plasticity and pluripotency, which can then be used to generate the appropriate cell type for that disease. For this reason, in the last decade, medical research is focused on the possible application of iPSC in regenerative medicine, using iPSC disease models for elucidating disease pathologies and drug discovery research.

To date there have been two main approaches for deriving sensory neurons from hPSC (Fig. 1). One approach is to recapitulate stages of DRG embryonic development in the culture dish, whereby hPSC are initially differentiated to neural crest progenitors followed by their subsequent differentiation to DRG neurons. The second approach is to direct hPSC differentiation to mature functional sensory neurons, bypassing the progenitor stage. Both systems have their pros and cons, which are outlined below.

2.1.1. HPSC differentiation to mixed populations of DRG sensory neurons

Developmentally, all DRG neurons arise from neural crest progenitor cells that migrate from the embryonic dorsal neural tube to the