Journal & Proceedings of the Royal Society of New South Wales, vol. 157, part 1, 2024, pp. 146–147. ISSN 0035-9173/24/010146-02

Thesis abstract

Specification of dorsal root ganglia sensory neuron subpopulations derived from human pluripotent stem cells

Amy Jane Hulme

Abstract of a thesis for a Doctor of Philosophy, submitted to the School of Medical, Indigenous and Health Sciences, University of Wollongong

The detection of sensations is essential for everyday functions and requires specialised dorsal root ganglia (DRG) sensory neurons to detect and transmit the stimuli to the central nervous system for processing. The DRG sensory neurons can be broadly classified as either (1) proprioceptors (that detect movement, muscle pressure, and tension), (2) low-threshold mechanoreceptors (LTMRs) (that detect touch, hair deflection, and vibration) or (3) nociceptors (that detect pain arising from harmful thermal, mechanical, and chemical stimuli). Unfortunately, there are major challenges in studying sensory perception and disease, including the difficulty in acquiring human tissue samples and the limitations in the translatability of rodent models due to inherent differences between human and rodent sensory neurons. The use of human pluripotent stem cells (hPSCs) can circumvent these challenges by providing a constant source of human cells that can then be differentiated towards sensory neuron cultures. However, current protocols to generate sensory neuron cultures are often limited by low reproducibility, low neuronal yields, mixed populations of neurons, prevalence of nonneuronal cells within the cultures, as well as the requirement of long maturation stages to obtain functionally mature neurons. A promising approach to generate populations of functional sensory neurons is by mimicking sensory neurogenesis using a combined stepwise addition of extrinsic factors (small molecules and growth factors) to direct hPSCs towards progenitor states and neuronal types, combined with the induced expression of lineage-specifying transcription factors to drive the differentiation to a specific neuronal fate. Thus, the major aim of the work described in this thesis was to derive DRG sensory neurons using a combined extrinsic-factor and induced-transcription-factor differentiation approach to generate cultures of sensory neurons and to then functionally characterise the sensory neurons.

A key goal of this PhD thesis was to mimic sensory neurogenesis by inducing the expression of lineage-specific transcription factors at a developmentally relevant progenitor cell type (i.e., enriched neural crest cells). The work presented in Chapter 3 describes the successful differentiation of hPSCs into caudal neural progenitors (CNPs), which were then further differentiated and enriched for neural crest cells. This protocol was then implemented in Chapters 4 and 5, which aimed to generate and functionally characterise hPSC-derived sensory neurons by inducing the expression of lineage-specific transcription factors in the hPSC-derived neural crest

cells. The work in Chapter 4 determined that the induced expression of the transcription factors, NEUROGENIN-1 (NGN1) or NEUROGENIN-2 (NGN2), in neural crest cells both significantly enhanced sensory neuron differentiation efficiency and generated a heterogeneous population of functional sensory neurons. The results presented in Chapter 5 demonstrated that the induced co-expression of the lineage-specific transcription factors, NGN2 and RUNT RELATED TRANSCRIPTION FACTOR 3 (RUNX3) or NGN2 and SHORT STATURE HOMEOBOX 2 (SHOX2) in hPSC-derived neural crest cells generated enriched mature sensory neuron cultures that had expression and functional profiles consistent with proprioceptors or LTMRs, respectively. Additionally, the work described in Chapter 5 also aimed to investigate whether there are functional differences in the mechanosensory physiology between the two classes of hPSC-derived mechanosensory neurons and the molecular mechanisms by which the two classes of hPSC-derived mechanosensory neurons respond to stimuli. The mechanosensory neurons, denoted as induced-proprioceptor neurons (iPN) and induced-LTMR neurons (iLTMR) were exquisitely sensitive to mechanical stimuli and exhibited distinct mechanically sensitive responses to stretch and to submicrometre (0.1 μm) mechanical stimulation by probe indentation to the soma. Additionally, the iPN and iLTMR displayed different adaptation kinetics reflective of distinct sensory specialisations. Importantly, the iPN and iLTMR fired action potentials in response to < 1.0 μm mechanical stimulation (probe Indentation) and knockdown experiments demonstrated that these responses to mechanical stimulation were predominately mediated by PIEZO2. Taken together, the work described in this thesis demonstrates the successful generation of heterogenous and enriched populations of functional sensory neurons from hPSCs via the combination of extrinsic factors and induced expression of lineage-specific transcription factors. The derived sensory neurons represent excellent models for the study of human sensory neuron development, peripheral neuropathies, mechanosensory physiology and for the development of directed therapies toward these neuronal populations that become compromised by trauma or neurodegenerative conditions.

Dr. Amy Jane Hulme
School of Medical, Indigenous and Health Services
University of Wollongong

E-mail: ahulme@uow.edu.au

URL: https://ro.uow.edu.au/theses1/1600/