Human Cells and Tissue in Pre-Clinical Studies: Human DRG

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Pain is a sensory and emotional experience most commonly initiated in response to a noxious stimulus. A group of peripheral sensory neurons, termed nociceptors, are the first neurons activated by noxious stimuli. The cell bodies of sensory neurons reside in the trigeminal ganglia (TG) or dorsal root ganglia (DRG), situated bilaterally and adjacent to the brainstem or vertebral column. These pseudounipolar neurons give rise to a peripheral branch that innervates target organs, for example skin and viscera, and a central branch that terminates in the brainstem or spinal cord dorsal horn [4]. Sensory neurons are highly heterogeneous, with multiple subpopulations, each possessing a constellation of response properties to non-nociceptive and nociceptive stimuli. The nociceptive neurons are not only responsible for signaling the presence of acute tissue injury, but appear to play an essential role in the ongoing pain and hypersensitivity associated with many chronic pain states. Thus, understanding the molecular and cellular underpinnings of signal transmission in and across nociceptors is essential for the development of effective and safe treatments for pain.

Experimental platforms to study pain

While it is ethically permissible to conduct some pain studies on human volunteers, these studies are narrow in scope, generally involve healthy individuals, and are rarely amenable to experimental interventions designed to reveal mechanisms. As a result, the mechanistic understanding of nociception and pain has been largely derived from the study of animal models in general, and mice and rats in particular [23]. Although many pathophysiological mechanisms are conserved between rodents and humans, important differences at the anatomical, molecular and cellular levels have very likely contributed to translational failure of some investigational pain medicines. These foundational gaps are starting to be bridged using human

nervous system tissue. Use of human nervous system tissues have potential to improve our understanding of the molecular and cellular basis of nociception and its modulation in humans. They will also serve as a cellular platform to interrogate the translational value of targets and drugs prior to embarking on costly human clinical trials [26]. Therefore, we will focus on studies on human DRG neurons in this fact sheet.

Nociceptors, receptors, ion channels, and pain

By necessity, nociceptors are normally quiescent and limit responses to noxious stimuli. However, these neurons may become sensitized. Sensitization is characterized by the emergence of spontaneous activity, a lowered threshold for activation, and/or an increased response to the same stimuli under a variety of conditions including nerve injury, tissue inflammation, and metabolic or genetic disorders, contributing to and likely causing chronic pain [5]. Specialized receptors found at the nerve endings of nociceptors are activated by noxious stimuli, leading to generator potentials that initiate nerve impulses in the form of action potentials, which are generated by an ensemble of voltage-gated ion channels. These action potentials propagate from the periphery to the spinal cord, where neurotransmitters are released from axonal ends. Subsequently, the signal is transmitted to spinal cord neurons and then to brain centers, where the signal is interpreted as pain [4]. Abundant evidence supports the notion that sensitization may be due to changes at any of these steps, from signal transduction to transmitter release. One obstacle to targeted treatment is that the cellular processes underlying sensitization appear to vary depending on the type of injury, site of injury, time following injury, sex, previous history, and genetics – including possible species differences between animal models and humans. Understanding the molecular basis of excitability of human nociceptors and their sensitization is therefore critical for the development of new and more effective drugs for treatment of pain.

Human DRG neurons in the quest to elucidate pain mechanism

Much of what we know about molecular and cellular underpinnings of nociception has been gleaned from studying rodent DRG neurons. Earlier studies of human DRG neurons typically involved a limited number of DRG neurons due to the paucity of viable human tissue [2; 8; 36]. This is changing with the increase in human tissue recovery from organ donors or rapid autopsy and improvements in methods for isolating and maintaining neurons in culture for molecular and functional studies [6; 12; 37]. The use of human DRG has confirmed conservation of basic mechanisms of nociceptor response to stimuli in rodent models. However, it also uncovered important differences that challenge some concepts established in rodent studies as will be discussed next.

Species-specific differences in the transcriptome and cellular composition of DRG neurons

Using elegant morphological and functional analyses, several subpopulations of rodent DRG neurons have been linked to specific sensory modalities. More recently, the advent of new sequencing technologies has enabled studies at the single-cell level, and detailed interrogation of the DRG transcriptome has confirmed the identity of subgroups of DRG neurons that are responsible for specific sensory modalities in the mouse and primates [14; 30; 35; 41]. However, the demarcation of modality-specific sensory neuron subgroups in human DRG neurons is not aligned with model species in several potentially important ways [18; 20; 25; 27; 31; 32]. For example, human nociceptors lack the distinctive separation of peptidergic and non-peptidergic nociceptors that have been reported in mouse neurons, and the expression of the thermal receptor TRPV1, which is also activated by capsaicin (the spice in hot chili pepper), is more widespread in human than in rodent DRG neurons [31-33]. These findings require us to think carefully about the interpretation of functional experiments that rely on

manipulation of genetically-defined subpopulations of DRG neurons in mice, as the same cell-type demarcations are not fully conserved in human DRG. As information continues to emerge from such profiling studies on human DRG, this information needs to be integrated with data from other species to advance our ability to translate targets into therapeutics.

Species-specific differences in ion channel and receptor properties

Immediately relevant to the development of new treatments for pain, human DRG studies are showing distinct properties of ion channels and receptors that are targets for the development of analgesics. While the expression of ion channels and receptors that regulate neuronal firing in rodent and human DRG neurons is highly conserved, there are notable species-specific differences in biophysical and pharmacological properties of channels and receptors [8; 10; 12; 13; 16; 29; 36; 40].

Rodent DRG neurons have lower thresholds for action potential generation compared to human DRG neurons [6; 12], which suggests a divergence in the relative abundance or biophysical properties of ion channels from the two species. Hartung et al [13] noted species-specific differences in the magnitude and biophysical properties of high voltage activated calcium channels, which play an essential role in the neurotransmitter release at the first synapse in the dorsal horn of the spinal cord. Zhang et al [40] observed that sensitivity of sodium currents in human DRG neurons to the select sodium channel blocker tetrodotoxin is less than that in rodent DRG neurons. While these studies of human DRG are valuable for establishing a baseline of knowledge, there are examples where the results generated by different laboratories studying human DRG neurons are not entirely consistent, for example in biophysical properties of the sodium current that is resistant to tetrodotoxin [12; 40]. Interestingly, the most striking differences in the results reported in these two studies were associated with the biophysical properties of the tetrodotoxin resistant currents in rat DRG neurons, highlighting the potential impact of the heterogeneity of sensory neurons that were recorded on results generated. Another example is the difference in the

biophysical and pharmacological properties of ionotropic acetylcholine receptors in human DRG neurons, which are different from those found in either mice or rats [39]. This species-specific difference might explain why agonists for these receptors ultimately failed in clinical trials for the treatment of pain [9; 11; 24; 28].

In addition to voltage-gated channels and ionotropic receptors, G-protein coupled receptors (GPCRs) have also been explored for potential analgesics. Several studies have demonstrated some functional and anatomical differences of opioid, cannabinoid, and other metabotropic receptors between rodent and human DRG [1; 3; 7; 18; 19]. Following ligand-gated activation of GPCRs, investigating the degree of conservation in intracellular second messenger signaling mechanisms and signal-induced gene expression may be important avenues for validating functional translation of novel analgesics or uncovering novel mechanisms for nociceptor modulation across species.

As the availability and use of human DRG neurons expand and more data are collected by independent groups, a clearer picture of the range of differences will emerge. It is already apparent, though, that the species-specific differences in the expression and properties of channels and receptors are reshaping our views about the molecular and cellular basis of nociception [17].

Limitations and opportunities for translation of this knowledge into clinical practice

The data set from studies of human DRG neurons are still limited in number, and access to this tissue is not yet widely available. The tissue is mostly only available from organ donors, whom by definition have experienced a catastrophic event, or from surgical resections or rapid autopsy, and thus labeling these neurons as "normal" is debatable. Indeed, organ donors are selected to be free of infectious diseases, disorders, and cancers that are often responsible for chronic pain and can compromise transplants. However, studies of DRG neurons from patients with chronic pain conditions are emerging [15; 21], and the comparison to data from neurons that are collected from subjects without a diagnosis of pain will be informative. Importantly, the existing datasets are not comprehensive

enough to address heterogeneity among the donors based on a number of factors (some easily identifiable and some unknown, including both biological and historical differences), which likely contribute to inter-individual variability in response to noxious stimuli among humans. Thus, there is a need for additional and more extensive studies using human DRG neurons, including the collection of extensive information on the history of the donors. This can only be done as more tissue becomes routinely recovered and widely disseminated.

Species-specific differences in ion channel properties and pharmacology can also be informative for the development of novel therapies for pain treatment. For example, Walker et al [38] determined that human Nav1.7 channel, which is a major target for drug development to treat pain, is >200-fold more resistant than predicted to block by the neurotoxin saxitoxin, and identified two residues in the outer vestibule of the channel that are different between primate and non-primate Nav channels, and which underlie resistance to the toxin. Building on this information, a derivative of saxitoxin was developed as a Nav1.7-selective blocker for clinical testing as a pain therapeutic [22; 34].

Overall, species differences in the properties of ion channels and receptors would be expected to confer different firing properties of these neurons and their response to drugs, making it imperative that new drugs are tested on their targets in the appropriate human cells. Furthermore, a function-based association of subsets of human DRG neurons with specific sensory modalities will be needed in the quest to develop mechanism-based treatments for pain. With the growing access to human neurons and continued improvements in their characterization, we may realize improved efficiency in the translation of findings from animal models, and the identification of novel targets that could not have been anticipated from animal model studies.

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