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FACT SHEET

Human Cells and Tissue in Pre-Clinical Studies: Induced Pluripotent Stem Cells

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Pain is adaptive because it warns us of impending harm, but chronic pain is maladaptive and remains a global unmet medical need. Nociceptors are those sensory neurons specialized in the detection of noxious stimuli (those stimuli that can cause tissue injury). They normally have a high threshold for firing, but nerve injury, tissue inflammation or genetic disorders causes the sensitization of these nociceptors which can lead to chronic pain. Understanding the molecular basis of excitability of these neurons and how they respond to disease or injury is critical for the development of new and more effective drugs for treatment of pain. Equally importantly, pre-clinical testing of investigational therapies on their cognate targets in human neuronal background is essential since species-specific differences in the properties of these targets may exist.

Current Experimental Approaches to Study Pain

The successful development of novel treatments for pain has been hampered by multiple factors, which must be addressed to bridge the gap between the basic pre-clinical knowledge and its translation into the clinic. Animal studies have been invaluable for elucidating the role of specific molecules and circuits in the transduction and transmission of noxious stimuli, and for pre-clinical in vivo pharmacology studies [27], but pain testing paradigms have been recognized as one impediment for the successful development of a clinically-useful drug [36]. Novel paradigms for testing pain behavior in animals have been developed for more translationally-relevant outcomes [31; 40]. Another impediment is the species-specific differences at the molecular and functional levels of several targets of new investigational drugs [5; 11-13; 16; 29; 33; 41]. Thus, testing of investigational drugs in human cellular models is a 'bridge' to translation [28].

Sensory Neurons Derived from Induced Pluripotent Stem Cells

Using human cells, including DRG neurons, in pre-clinical studies of investigational drugs has the advantage of assessing target engagement and efficacy of drugs in native cellular background. Although there is growing interest in and adoption of using human DRG neurons in cell-based assays, it is still difficult to obtain native DRG neurons on

a large scale for wide-spread use in pre-clinical studies [28] and because these are post-mitotic they are not amenable to large scale genome engineering or expansion. The development of an approach to differentiate sensory neurons from iPSC (iPSC-SN) [2] has provided a technology platform for understanding disease pathophysiology including the mechanisms of neuronal injury and hyperexcitability, development of cell-based assays to screen and validate novel drugs prior to embarking on costly clinical trials, and for the identification and validation of biomarkers, or for the iPSC-SN to serve as biomarkers of disease *per se* [22; 32].

iPSC-SN to Study Disease Mechanisms

iPSCs retain the genetic background and native transcriptional machinery of individuals [14; 30; 39], thus allowing mechanistic studies of disease that are not subject to potential artifacts that could arise from over-expression systems or transgenic animal studies. Patient-specific iPSC-SN provide a "disease-in-a-dish" model of pain, with the best examples coming from studies of Nav1.7-related pain disorders inherited erythromelalgia (IEM) and congenital insensitivity to pain (CIP) [1; 19; 21; 23; 38]. Studies have shown that patient-specific iPSC-SN carrying gain-of-function mutations in Nav1.7 are more excitable than those from control subjects [1; 21; 23], consistent with the enhanced pain that is experienced by carriers of these mutations. By contrast, iPSC-SN from patients with Nav1.7-related CIP showed lower excitability than that of control lines. Importantly excitability was normalized when one of the mutations was corrected using CRISR-Cas9 genomic editing [19]. Other examples of patient-specific iPSC-SN that are used to study mechanisms of pain include migraine [26] and a number of different types of neuropathy such as small fiber neuropathy (SFN) and Fabry disease [15].

SFN is frequently associated with adult-onset of a reduction in the number of intraepidermal nerve fibers in the skin and clinical symptoms of chronic pain of burning nature. It has been linked to variants in voltage-gated sodium channels [9], and iPSC-SN are a useful model to study the effects of these variants on neuronal excitability [25] and potentially study the link between cellular function and anatomical loss of nerve fibers. Hereditary sensory neuropathy type-1 (HSN-1) is a painful neuropathy particularly affecting small fibers which is due to heterozygous mutations in the gene *SPTLC*1. This leads to altered sphingolipid metabolism and the production of toxic Deoxysphingobases (DSBs). Elevated DSBs are detectable in human iPSCd-SN from HSN1 patients along with reduced production of complex gangliosides, axon degeneration and hyper-excitability. These were then used to test disease modifying therapy and addition of Serine which reduces DSB production could prevent many of these changes [3].

iPSC-SN for Personalized Medicine

The development of patient-specific iPSC-SN provides an opportunity to test the response of these neurons to specific drug treatments. The attenuation of excitability of patient-specific iPSC-SNs in a dish to a specific drug would suggest target engagement in vivo and the expectation that a patient may benefit from such a treatment, while a poor response of the neurons in vitro would suggest that treatment of the patient with this drug may not be effective. This is the essence of personalized medicine which reduce the need for a trial-and-error approach to treatment. There is a small number of cases using patient-specific iPSC-SN in which tenets of this personalized medicine approach appear to have been met [1; 25]. While this approach might be useful in small clinical studies, the generation and maintenance of iPSC-SN is labor intensive and expensive and thus needs to be scaled up and best be automated before it may be useful for widespread application in the clinic.

iPSC-SN: Genetic Substrate to Identify Novel Targets for Drug Development

Except for cases of CIP, pain response to a noxious stimulus is universal, however, the experience at the individual level is variable. This inter-individual variability in response to the same stimulus is related to genetic and epigenetic factors [4; 6; 17; 24; 42]. Parallels between pain and hyperexcitability of patient-specific iPSC-SN could provide an opportunity to discover modifier genes that contribute to the individual pain experience. Recent studies capitalized on the availability of well-phenotyped members of the same family with the painful disorder IEM due to the same Nav1.7 gain-of-function mutations S241T or F1449V, who manifest distinct differences in salient pain features [10; 20; 38] to establish and validate "pain-resilience-in-a-dish" model that is beginning to identify modifier genes that confer inter-individual differences in neuronal excitability which

possibly contribute to the inter-individual differences in pain symptoms in these subjects [23; 38]. Unbiased whole exome screening and in silico analyses identified variants in Kv7.2 and Kv7.3 in the resilient subject and functional testing provided evidence for a role of these variants in regulating excitability of iPSC-SNs supporting the presence of a peripheral component to pain resilience, and suggesting that targeting Kv7 channels can be an effective avenue for pain treatment. Although Kv7 channels are established targets in pain studies and Kv7.2/Kv7.3 channel openers attenuate nociceptive behaviors in animal models of pain [7; 35], additional modifier genes are undoubtedly involved in the integrated response of these subjects to the painful stimulus. This cell-autonomous approach may lead to the identification of additional modifier genes and thus novel targets for drug development.

Conclusion

Patient-specific iPSC-SN have provided a powerful cell-autonomous system to correlate excitability of these neurons with pain phenotype in patients, and for testing response to drugs or other therapeutic approaches, which enhances the value of this pre-clinical data for further clinical development of investigational reagents. However, iPSC-SN do not fully capture the heterogeneity of human DRG neurons [8; 22] despite a report that they share 80% of transcripts found in adult human DRG neurons [37]. Additionally, current differentiation protocols have not been successful in reproducibly generating the full complement of components of the electrogenosome that is present in native neurons [8]. Despite these limitations, *in vitro* studies of iPSC-SN and human DRG neurons (when available) have already advanced our understanding of the cellular basis of pain pathophysiology and promise to improve chances of successful translation of pre-clinical data on investigational drugs into clinical use. There are promising approaches to address these limitations and improve the utility of iPSC in pre-clinical studies. Co-cultures of iPSC-SN and iPSC-derived skin tissue will lead to an "organ-in-a-dish" experimental system. Other exciting advances include transplantation of human iPSC into rodent DRG in vivo [34] and growing DRG organoids [18], both of which to take advantage of the microniche environment to differentiate human sensory neurons in a more nativelike environment. Thus, we predict that the use of subject-specific iPSC represents significant advances in the field and will aid in bridging the translational gap between pre-clinical knowledge and the development of effective analgesics.

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