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Getting a Foot IN the Door: GABAergic INterneuron-Specific Enhancers

Viral Manipulation of Functionally Distinct Interneurons in Mice, Non-Human Primates and Humans

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Recent success in identifying gene-regulatory elements in the context of recombinant adeno-associated virus vectors has enabled cell-type-restricted gene expression. However, within the cerebral cortex, these tools are largely limited to broad classes of neurons. To overcome this limitation, we developed a strategy that led to the identification of multiple new enhancers to target functionally distinct neuronal subtypes. By investigating the regulatory landscape of the disease gene Scn1a, we discovered enhancers selective for parvalbumin (PV) and vasoactive intestinal peptide-expressing interneurons. Demonstrating the functional utility of these elements, we show that the PV-specific enhancer allowed for the selective targeting and manipulation of these neurons across vertebrate species, including humans. Finally, we demonstrate that our selection method is generalizable and characterizes additional PV-specific enhancers with exquisite specificity within distinct brain regions. Altogether, these viral tools can be used for cell type–specific circuit manipulation and hold considerable promise for use in therapeutic interventions.

Commentary

Single-cell analysis—including single-cell RNA sequencing and Assay for Transposase-Accessible Chromatin using sequencing (scATACseq)—facilitates the study of genomics and transcriptomics at the single-cell level and has been used in neuroscience for cell classification and the study of evolution, neurodevelopment, cell function, and neuropathology, among other applications. Recent large-scale transcriptomic studies of autism, schizophrenia, and other disorders indicate cell type–specific expression of disease-related genes.[1,2] It has been shown that defined subsets of neurons may be dysfunctional in distinct epilepsy syndromes (an example being parvalbumin (PV)-positive fast-spiking GABAergic interneurons—PV-INs—in Dravet syndrome[3,4]) and that specific subsets of neurons can be manipulated to terminate seizures in experimental model systems.[5,6] With the ongoing development of gene therapy for neurological disorders using recombinant adeno-associated viral vectors (rAAV), the identification of transcriptomic signatures of key epilepsy-related cell types and development of methods for cell type-specific targeting and manipulation of human neurons may become possible and could lead to advances in epilepsy therapy.

Cell type–specific Cre driver mouse lines allow for identification and targeted electrophysiological recording, imaging, and manipulation of genetically and molecularly defined cell types in experimental model systems such as mouse as well as other mammalian species including rat and marmoset. However, different approaches are needed for cell type–specific targeting in humans. Recombinant adeno-associated viral vector can have general predilection for tissue types (such as liver, skeletal muscle, etc) but are not inherently selective for a particular neuronal cell type. Use of regulatory elements can restrict expression to pyramidal cells (eg, CaMKIIα), INs (mDlx[7]), and defined subsets of INs such as somatostatin (SST)-expressing INs.[8]

Here, Vormstein-Schneider et al[9] studied the regulatory architecture of *Scn1a* which encodes the type 1 neuronal voltage-gated sodium channel α subunit Nav1.1. This gene is associated with Dravet syndrome and is known to have a cell type–specific expression pattern as noted above. The authors isolated nuclei from PV-INs and performed scATACseq to identify *Scn1a* regulatory sequences present in areas of chromatin accessibility. The authors used comparative genomics to select 10 candidate sequences conserved across mammalian

species (enhancer sequences denoted E1-E10) that were located close to the transcriptional start site of *Scn1a* and then screened these elements for ability to drive cell type–specific viral expression.

The authors identified the PV-specific enhancer E2 which was subsequently shown to facilitate specific targeting of PV-INs across species, including mouse, nonhuman primate, and in human tissue. Samples of adult human tissue were obtained from 4 temporal lobe resections performed for treatment of intractable epilepsy. This tissue was preserved post-resection, sliced in the laboratory, and maintained for 7 to 14 days in culture. Incubation with pAAV.E2.tdTomato labeled a subset of neurons that were confirmed to co-express PV; targeted whole-cell current clamp electrophysiological recording from tdTomato-positive human neurons in vitro demonstrated that these cells were indeed fast-spiking cells. Use of E2 to target fast-spiking PV-INs in human tissue was critical toward supporting the potential translational relevance of these findings.

The authors also used rAAV.E2 in experimental animals to target synaptophysin-tdTomato to PV-INs to label PV-IN synapses; to target the genetically encoded calcium indicator GCaMP6f to PV-INs for in vivo 2-photon calcium imaging; the chemogenetic actuators PSAM4 and hM3Dq-DREADD for chemogenetic inhibition of PV-IN activity; and the excitatory opsin C1V1 for optogenetic control of PV-INs.

An interesting side question raised by the authors is whether a subset of cases of the *SCN1A*-related spectrum of disorders (including Dravet syndrome) could be due to pathogenic variants in or microdeletions of these enhancer elements, particularly the E2 enhancer. Such variants might lead to loss of *SCN1A* specifically in PV-INs which could lead to Dravet syndrome or a Dravet-like syndrome given the known role of PV-IN dysfunction in Dravet syndrome pathogenesis.

Overall, these findings—that E2 labels PV-INs across species including in human tissue and can be used to deliver chemogenetic agents or optogenetic actuators to PV-INs—are critical evidence in support of the potential utility of this tool for targeting and manipulating the activity of PV-INs in human patients for the treatment of epilepsy. Future application to humans is further supported by a recent paper confirming that the PV-IN transcriptomic cell subtype is conserved across evolution,[10] which lends confidence to the idea that PV-INs constitute a distinct cellular entity with a presumed similar functional role across species.

In a separate paper published shortly thereafter, Chen et al[11] used this E2 enhancer to drive expression of the red-shifted channelrhodopsin ChRmine in PV-INs to terminate seizures in the intrahippocampal kainate model of chronic temporal lobe epilepsy in mice. "Deep brain optogenetics" using red light to activate a red-shifted channelrhodopsin through the skull without the need for a craniotomy was more efficient when PV-INs were targeted using E2.ChRmine than when INs were targeted with mDlx.ChRmine.

Vormstein-Schneider et al also show that the E6 regulatory element is highly selective for GABAergic INs expressing vasoactive intestinal peptide (VIP-INs). VIP-INs are another prominent subset of GABAergic INs in the cerebral cortex which are considered to serve a "disinhibitory" role in microcircuits of neocortex and hippocampus via inhibition of SST-INs. Identification of a VIP-IN-specific enhancer of *Scn1a* is consistent with the recent discovery that VIP-INs in fact express Nav1.1 and that a subset of VIP-INs are dysfunctional in *Scn1a*+/− mice.[12]

Caution is certainly warranted here, and further development and validation of this technology is required prior to consideration of any potential use in human patients. The specific AAV vectors would need to be shown to be safe. Such vectors would need to demonstrate the necessary widespread infection if administered systemically or intrathecally, which has previously been a barrier in the field; otherwise, delivery would need to be via craniotomy and focal or multifocal injection. Expression of optogenetic actuators such as ChR2 variants via rAAV.E2 would need to be shown to achieve stable expression over long periods of time without cellular toxicity. And while E2 was highly specific for PV-INs in adult tissue, it was less specific for PV-INs when injected at early developmental time points, which could restrict the applicability of this tool in the pediatric population where it might be most relevant.

Despite such concerns, this work reports an exciting new tool that may be useful for the selective and widespread targeting of PV-INs across species, giving us a foot "IN" the door toward doing so in humans. Informed by comparative genomic information on cell types,[10] this general approach could potentially be used to identify cell type–specific regulatory elements to unlock the capability to selectively target, record, and manipulate any genetically defined cell type. With approaches such as "deep brain optogenetics," it is becoming easier to envision the combined application of rAAV technology to deliver optogenetic actuators or other effectors to specific cell types for the treatment of neurological disease such as epilepsy. For example, one could envision targeted expression of an excitatory opsin to PV-INs and an implanted or external device for light delivery that would recruit PV-IN activation "on-demand" based on the detection of a particular EEG signature, movement pattern, or in response to a patient's own subjective experience.

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