NEUROSCIENCE

Generation of AAV vectors for selective neuronal targeting

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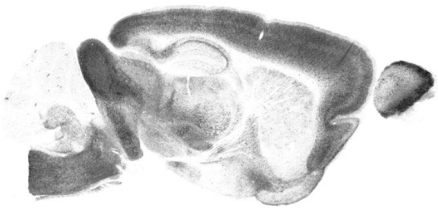
Many genes associated with neuropsychiatric diseases are expressed by specific neuronal subtypes. Approaches to selectively target these cell populations within the brain are lacking, hampering the development of targeted gene therapies. In a new study published in *Nature Neuroscience*, a team of investigators led by Jordane Dimidschstein from the Broad Institute of Harvard and MIT describes a strategy to generate several recombinant adeno-associated virus (rAAV) for selective targeting and manipulation of neuronal subtypes, which holds promise for future therapeutic interventions.

The viral tools were built by integrating DNA regulatory sequences—enhancers identified in the locus of a disease gene expressed in specific neurons—in rAAV backbone vectors. The resulting rAAV vectors were used to target and manipulate the neuronal subtypes in the cortex of adult mice. "Ultimately, these may provide the means to therapeutically normalize pathological neuronal activity or gene expression in specific neuronal populations," explain the investigators in their report.

The team focused on *Scn1a*, a gene that has been associated with Dravet's syndrome, a form of epileptic encephalopathy characterized by early onset of seizures. *Scn1a* is expressed in three neuronal populations: fast-spiking cortical interneurons (cIN) expressing parvalbumin (PV), disinhibitory cINs expressing the vasointestinal peptide (VIP cINs) and layer 5 pyramidal neurons. To identify enhancers regulating the expression of the gene in these different neuronal subtypes, the team used an integrative method combining data from single-cell assay for transposable-accessible chromatin sequencing (scATAC-seq) on mouse brain samples with sequence conservation analysis across species. They identified ten chromatin-accessible enhancers (active enhancers) showing high conservation across mammalian species, including humans.



Sagittal section of brain from adult mouse that was injected systemically with rAAV-E2-dTomato and analyzed 3 weeks post-injection with IHC for the viral reporter. Scale bar represents 500 μm; Reprinted with permission from Vormstein-Schneider et al. (2020) Springer Nature.

The sequences were inserted into an rAAV backbone containing a minimal promoter upstream of a red fluorescent reporter (rAAV-E[x]-dTomato). rAAVs were then produced and systemically injected into adult mice, which were analysed after 3 weeks for dTomato expression. The results showed that among the different enhancers, E2, E6 and E5 were able to restrict viral expression to PV cINs, VIP interneurons and pyramidal neurons in layer 5, respectively.

"These data indicate that a large fraction of the neuronal populations expressing *Scn1a* in the cortex is mirrored by the collective expression of these three enhancers. Notably, these regulatory elements account for largely nonoverlapping expression in populations of neurons with distinct functions and developmental origins," write the investigators.

Next, the team demonstrated that E2—the enhancer with >90% selectivity for PV cINs—could be used to selectively deliver different chemogenetic and optogenetic tools to PV cINs and modulate their activity ex vivo and in vivo in the cortex of adult mice.

To establish whether E2 could be used to target PV cINs across mammalian species, rAAV-E2 vectors driving a fluorescent reporter were systemically injected in marmosets or focally in rats and macaques. Analysis of the animals 2–8 weeks after injection revealed that E2 targeted PV cINs with 90% specificity across all three species. Similar results were obtained ex vivo on human brain slices.

Next, the investigators applied their enhancer selection method to identify more enhancer candidates in the locus of seven genes enriched in PV cINs. Systemic injection of rAAVs containing these sequences in adult mice revealed that four of them selectivity targeted different subsets of PV cINs, which confirmed the generalizability of the method to identify cell-specific enhancers.

These findings might have several clinical implications. The enhancers identified in the study could be used to develop gene therapy approaches to alleviate debilitating aspects of Dravet's syndrome. The enhancer selection method could also be utilized as a tool for systematic identification of cell-type-specific enhancers.

"The combination of regional selectivity and conservation of expression across species demonstrates the scalability of the enhancer selection method. This opens the possibility of using these tools for work in non-human primates and paves the way for the development of targeted therapies to correct abnormal brain function," say the investigators in their report.

Alexandra Le Bras

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