Developing direct in vivo genome editing and screening platforms in the mouse brain

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The neuropsychiatric effect of nicotine is under the delicate control in the brain. Despite rapid progress in the field, it is still challenging to understand such complex interactions in vivo, largely enormous neuronal, genetic and epigenetic complexity. We proposed to tackle this problem by developing enabling platforms to precisely and systematically interrogating the neurogenetic circuits in the brain. We have recently developed an adeno-associated virus (AAV)-mediated autochthonous CRISPR direct in vivo screen technology for highly multiplexed brain gene editing and genetic screening. We piloted this system for study of human cancer as a proof-of-principle. Stereotaxic delivery of an AAV library targeting genes commonly mutated in human cancers into the brains of conditional Cas9 mice resulted in tumors that recapitulate human GBM. Capture sequencing revealed diverse mutational profiles across tumors. The mutation frequencies in mice correlate with those in two independent patient cohorts. Co-mutation analysis identified co-occurring driver combinations such as Mll2, B2m-Nf1, Mll3-Nf1 and Zc3h13-Rb1, which were subsequently validated using AAV minipools. Using a well-established nicotine addiction paradigm, we recently plan to apply this AAV-CRISPR system for neuronal manipulation in nicotine circuit with barcoding capacity. These new platforms and insights will facilitate the study of genetic and epigenetic basis of nicotine exposure, which can be broadly applicable to other neuropsychiatric diseases and drug addiction, ultimately leading to development of better therapeutic strategies for substance use disorders.