Massively parallel single-nucleus transcriptomic profiling to identify dysregulated gene regulatory circuitry in opioid addicted human brain

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Substance use disorder is a major threat to public health that affects millions of people worldwide and imposes devastating social and economic impacts on individuals and their families. The addiction phenotype usually persists years after drug abuse and very often relapse even after years of absence from the drug. The life-long persistence of the addiction phenotype suggests that chronic drug usage triggers long-lasting alterations in the brain that causes the addictive behavior. Increasing evidence shows that chronic exposure to abusive drug changes gene expression through altered epigenetic regulation and potentially could contribute to the long-lasting persistence of drug addiction. However, brain is the most complex organ in our body and is composed of many different neuronal and glial cell types and each with distinct functions. The underlying molecular mechanism of addiction is still largely unknown, in part because the process is very complex and because multiple cell types are involved. We propose to utilize massively parallel single-cell transcriptomic methodologies to dissect dysregulated gene circuitry for each of these different cell types in the brain of opioid dependent individuals and matching drug-free controls. This will enable a systems level understanding of the molecular alterations in substance addiction. Our preliminary results show the feasibility of applying massively parallel droplet based single nucleus RNA-seq on postmortem human brain tissue. By profiling 1827 single nuclei from frozen postmortem brain tissue in the nucleus accumbens, we were able to identify and characterize gene expression profiles in each of the major cell types in the human brain such as glutamatergic neurons, GABAergic neurons, astrocytes, microglia, oligodendrocytes and oligodendrocyte precursor cells. We are in the process of characterizing nucleus accumbens from opioid addicted individuals and drug free controls. By understanding the dysregulated gene circuitry of substance addiction, our ultimate goal is to discover potential molecular diagnostic markers and identify novel therapeutic targets.