Proteomics Signatures of Drug Perturbations in Neurobiological Model Systems

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Neuropsychiatric Genome-Scale and RDoC Individualized Domains (N-GRID) project

Abstract

Changes in the epigenetic and phosphosignaling landscape occur through different stages of neural development, and play an important role in the etiology of many neurological disorders such as autism spectrum disorders (ASDs). To examine the role and global-scale connections between the phosphoproteome and epigenetic states in neural cells, we are leveraging P100 phosphoproteomic profiling and global chromatin profiling (GCP) to characterize healthy neural progenitors and functionally mature neurons. We also aim to apply the CRISPR-Cas9-mediated genomic editing technology to disrupt the expression of core epigenetic factors, and to introduce gene variants associated with ASD into human stem cells. Our goal is to combine these P100 and GCP profiles with those of known active drugs in neural cells to identify new therapeutic avenues in ASD and related disorders. We have successfully derived neural progenitor cells (hNPCs) from human pluripotent stem cell lines, and present our progress in optimizing the CRISPR-Cas9 system and in producing functionally mature neurons. In addition, we present P100 and GCP data from hNPCs in response to 90 pharmacologically active small molecules, and after commitment to a neural lineage.

Figure 1. The plan

Core Epigenetic Reference Genes

CHD8	HDAC2	KDM2A	KMT2A	SIRT6	BRD9
DNMT1	HDAC3	KDM2B	KMT2D	SIRT7	CBX1
DNMT3A	HDAC4	KDM3A	KMT2C	SUV39H1	CBX2
DNMT3AP1	HDAC5	KDM3B	KMT2E	SUV39H2	CBX3
DNMT3B	HDAC6	KDM4A	SETD1A	SUV420H1	CBX4
DNMT3L	HDAC7	KDM4B	SETD1B	SUV420H2	CBX5
DOT1L	HDAC8	KDM4C	SETD2	WHSC1	CBX6
EHMT1	KAT2A	KDM4D	SETD7	WHSC1L1	CBX7
EHMT2	KAT2B	KDM4E	SETD8	NELFA	CBX8
EZH1	KAT5	KDM5A	SETDB1	ASXL1	EED
EZH2	KAT6A	KDM5B	SETDB2	BRD1	EP300
HAT1	KAT6B	KDM5C	SIRT1	BRD2	NSD1
HDAC1	KAT7	KDM5D	SIRT2	BRD3	SMYD1
HDAC10	KAT8	KDM6A	SIRT3	BRD4	SMYD2
HDAC11	KDM1A	KDM6B	SIRT4	BRD7	SMYD3
SUZ12	KDM1B	KDM8	SIRT5	BRD8	CLOCK

Autism-Spectrum Disorder & Other Neurobiology Genes

ADNP	DYRK1a	MECP2	SEMA5A
ARID1B	EHMT1	NLGN3	SHANK3
CACNA1C	EIF4G1	NLGN4X	SYNGAP1
CNTN4	EPC2	NRXN1	TLK2
CNTNAP2	FMR1	PTCHD1	TSC1
CREBBP	GRIN2B	PTEN	TSC2
CTCF	GRIP1	SCN1A	UBE3a
CTNNB1	GRM5	SCN2a	UBE3b

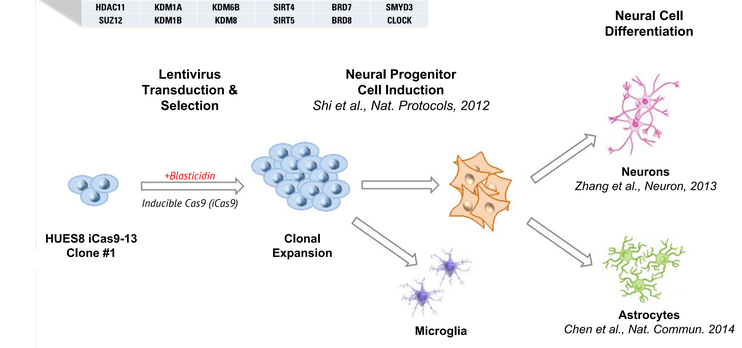


Figure 2. A) Expression of Cas9 can be induced with doxycycline. B) Lentiviral transduction of neural precursor cells

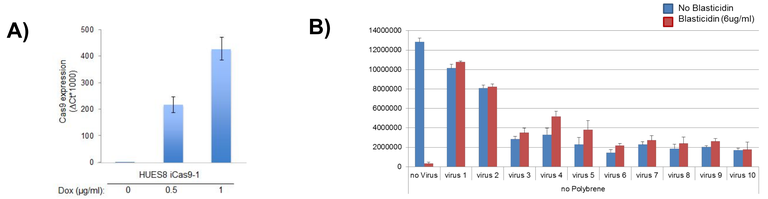


Figure 3. Protocol delivers mature neurons mixed with neural precursors and glia

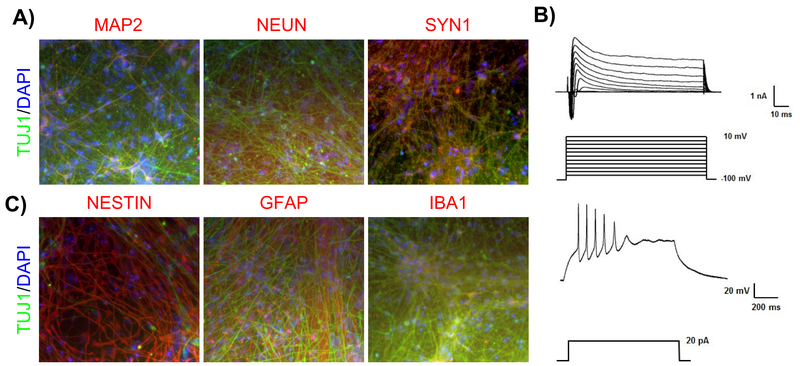


Figure 4. Strategies for promoting efficient neural differentiation

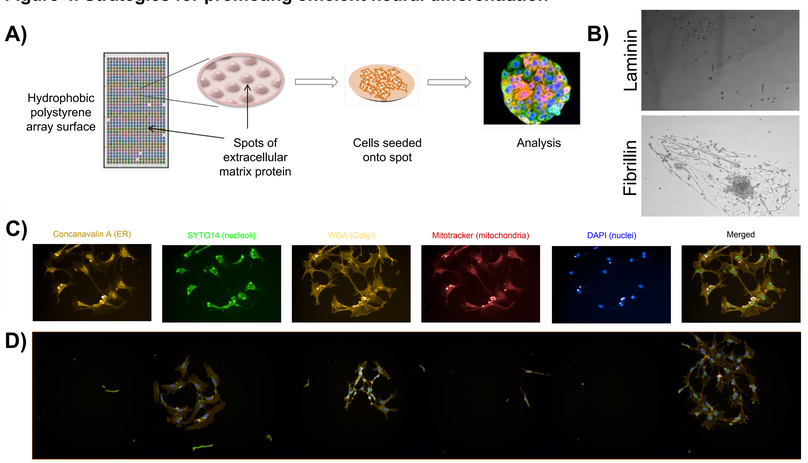


Figure 5. The phosphoproteome in neural cells are unique

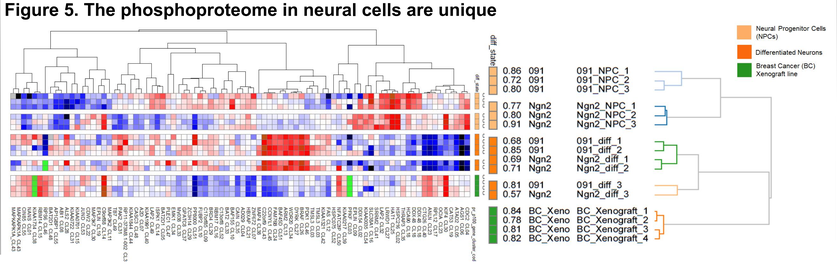
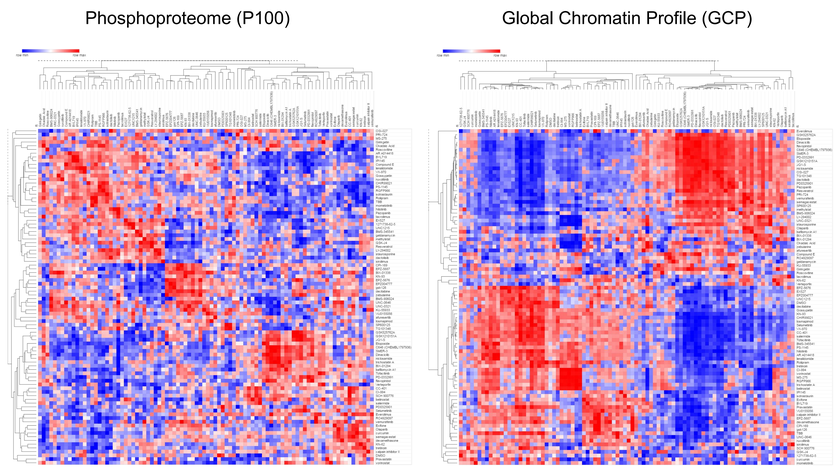


Figure 5. Profiling drug responses in neural precursor cells



Next steps

- Optimize differentiation protocol to yield mature neurons with a minimal number of neural precursors and glia
  - Time course with mitotic inhibitor Cytosine β-D-arabinofuranoside (AraC) to determine dosing regime
  - Screening MEMA arrays to identify substrates the promote more efficient differentiation (collaboration with MEP-LINCS at OHSU)
- Profile phosphoproteome and chromatin response of 90 pharmacologically compounds in neurons
- Test Cas9 knock-out system with pilot set of sgRNAs
- Scale up for screen in NPCs and neurons
- Examine how loss of ASD-related genes impacts phosphoproteome and chromatin state of neurons and NPCs
- Can we rescue deficits associated with ASD?

References

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