Characterizing Clinical Variants of the Neurodevelopmental Disorder-associated Enzyme KMT5B

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Background: Neurodevelopmental disorders (NDDs) are estimated to affect up to 15% of children and adolescents worldwide; however, the underlying pathology of many NDDs is poorly understood. Lysine methyltransferases - the writers of lysine methylation - have been implicated in numerous neurodevelopmental disorders. The lysine methyltransferase KMT5B has a catalytic SET domain that dimethylates lysine 20 of histone H4 as well as non-histone substrates, and KMT5B haploinsufficiency has been implicated in NDDs. Clinical variants of KMT5B have been associated with autism spectrum disorder, intellectual disability, developmental delays, macrocephaly, and Chiari malformations; however, the impact of variants found in patients on KMT5B's methyltransferase activity is not known.

In this work, we aim to 1) characterize the impact of clinical variants of KMT5B on its methyltransferase activity and 2) begin preliminary work on assessing the heterogeneity of cells undergoing single-cell sorting for future experiments to introduce clinical variants of KMT5B into neuronal cells.

Methods: Recombinant reference and variant forms of KMT5B were overexpressed and purified from *E. coli.* Enzymatic activity was assessed using a radiation-based KMT assay with mononucleosome substrate. Neuronal differentiation was monitored by live cell imaging using IncuCyte NeuroTrack software.

Results: *In vitro* assays displayed a varied decrease in enzymatic activity in variants T85I, C200R, L263F, and E302K. Interestingly, the variant S73F showed an increase in KMT activity, despite the patient displaying the phenotype typically associated with haploinsufficiency.

Potential Impact: The clinical variants S73F, fsA74PTer10, C200R and L263F will be introduced into a neuronal cell model to assess the impact on differentiation. Characterization of these variants in a differentiation model will help elucidate whether there is a potential role of KMT5B catalytic activity in neuronal development.