

# Decoding ASXL3: A Novel Biomarker for Neurodevelopmental Disorders

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In a recent study, 33% of individuals with neurodevelopmental disorders (NDDs) carried mutations in ASXL3, whose function is unknown. To understand ASXL3's role in NDDs, we used CRISPR technology to engineer three H9 embryonic stem-cell lines with varying doses ASXL3 levels: wild-type (ASXL3 +/+), heterozygous (ASXL3 +/-), and homozygous knockout (ASXL3 -/-). PCR and DNA sequencing confirmed that CRISPR-CAS9 generated the intended cell lines. Using these newly engineered cell lines, we generated rosettes and organoids to track cell growth and characterize cell differentiation through imaging and RNA sequencing analysis. The ASXL3 -/- cell lines exhibited accelerated growth compared to ASXL3 +/- or ASXL3 +/+. Furthermore, ASXL3 -/- organoids had an increased percentage of proliferative cells, evidenced by increased number of Ki67 cells, leading to an increase of neural progenitor cells. However, ASXL3 -/- organoids had reduced neuronal differentiation and less layer 5 cortical neurons, characterized by fewer BCL11B cells. This revealed the novel function of ASXL3 as a neural cell gate control. Additionally, the less layer 5 neurons impair the layer 5 functions of communication and fine motor skills, which generates the phenotype of the NDD, showing that ASXL3 -/- is a novel biomarker for NDDs. This pattern of increased proliferation and reduced differentiation was independently verified by differential RNA sequencing analysis between ASXL3 +/+ and ASXL3 -/-. Finally, to rescue the defects observed in ASXL3 -/- cells, we treated them with fibroblast growth factors (FGF) at 10, 20, and 30 ng/ml concentrations and found that 30 ng/ml of FGF in an ASXL3 -/- promoted neuronal cell differentiation similar to ASXL3 +/- in 92% of our cells and may alleviate the effects of NDDs.

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