A novel *in vitro* platform for rapid generation of CRISPR Knockout in pluripotent stem cell derived human neurons.

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Human pluripotent stem cells (PSCs) technology combined with CRISPR/Cas9 mediated gene editing is a very powerful tool in understanding the molecular basis of human physiology. Human neurons can be differentiated from PSCs to study the physiological and pathological conditions of the human central nervous system (CNS). Furthermore, it has been shown that human CNS neurons can be rapidly differentiated from PSCs by over-expressing the neurogenin2 (Ngn2) gene. However, CRISPR/Cas9 delivery to these human CNS neurons can be inefficient and toxic. In the current study, we have generated a dual targeted human PSC line (H1-Cas9-Ngn2) that contains doxycycline (dox)-inducible transgenes for Cas9 and Ngn2, introduced into the AAVS1 "safe-harbor" loci of the H1 human embryonic stem cell line. Upon dox induction, we observed significant increase in Ngn2 and Cas9 expression. The rapid induction in Ngn2 expression coincided with increased expression of neuronal marker MAP2 and decreased expression of pluripotency marker NANOG. Indeed, we observed accelerated development of neuronal morphology and found that more than 90% of the culture were immuno-positive for MAP2 confirming that Ngn2 expression led to quick and homogenous generation of CNS neurons. We next demonstrated that dox-induced Cas9 expression is sufficient to mediate genome editing, by transducing the PSCs with single guide RNA targeting the PTEN gene. DNA sequence analysis confirmed that CRISPR/Cas9-mediated gene editing led to PTEN indel efficiency of more than ~95%. Western blot analysis confirmed the ablation of PTEN protein and significant increases in the phosphorylation of downstream targets AKT and S6. We found that these PTEN mutant neurons displayed increased neuronal soma size and neurite arborization. Using multi-electrode arrays and patch clamp recording, we found that PTEN mutant neurons are hyper-excitable and hyper-synchronized. Using microfluidic devices that separate the neuronal cell bodies and their axons, we demonstrated that PTEN mutant neurons displayed enhanced axon regeneration upon axotomy. Beyond this proof-of-principle study focused on the PTEN gene, this PSC-based in vitro platform is ideally suited for rapid CRISPR knockout screens to identify novel CNS neuronal phenotypes.