Altered calcium homeostasis and PTEN-related signaling in human iPSC-derived neural progenitor cells from Huntington's disease patients

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iPSC Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expanded CAG repeat in the HTT gene. Although classically associated with adult-onset striatal neurodegeneration, increasing evidence suggests that mutant huntingtin may also disrupt early neurodevelopment. Because huntingtin is essential for embryonic development and neural maturation, we investigated how mutant huntingtin affects human neural progenitor cell (NPC) biology. Human induced pluripotent stem cell-derived NPCs (hiPSC-NPCs) from HD patients and healthy donors were evaluated for GABAergic differentiation, calcium homeostasis, cell death, proliferation, and PTEN-associated signaling. NPCs expressed Nestin and SOX2 and were differentiated using twoand three-step protocols, generating cells positive for NeuN, TUJ1, Synaptophysin, and GAD65/67, establishing a human model for studying HD-related neurodevelopmental alterations. HD-derived NPCs showed altered colony morphology, attachment, and cellular status compared with controls. Flow cytometry revealed trends toward increased SOX2 expression and Ki-67 positivity, suggesting altered progenitor maintenance and proliferation. HD NPCs also exhibited increased Annexin V staining and more propidium iodide-positive detached cells, indicating enhanced cell death. In contrast, activated caspase-3/7 levels were reduced, suggesting involvement of cell-death mechanisms not primarily dependent on classical caspase-3/7-mediated apoptosis. FLUO-4 calcium imaging revealed impaired spontaneous calcium activity in HD NPCs, characterized by fewer spike-like oscillations and predominance of wave-like patterns, potentially associated with elevated basal calcium levels. Because calcium oscillations regulate neural differentiation and survival, these alterations may disrupt calcium-dependent developmental programs. We further investigated PTEN, a regulator of PI3K/Akt signaling and phosphoinositide metabolism. Pharmacological PTEN inhibition with VO-OHpic and depletion of endoplasmic reticulum calcium stores with thapsigargin produced greater effects in HD NPCs, including reduced Nestin and SOX2 expression, consistent with induction of differentiation. Together, these findings indicate that mutant huntingtin alters human neural progenitor biology by disrupting calcium homeostasis, survival, proliferation, and PTEN-associated signaling. They support an early neurodevelopmental component of HD and identify calcium- and PTEN-related pathways as potential mechanisms contributing to disease vulnerability before overt neurodegeneration.