Human IPS cell models of cholesterol synthesis disorders reveal WNT signaling defects underlie neurological dysfunction

Kevin Richard Francis

NIH/NICHD, Bethesda, MD, USA

Background and objectives: Autosomal recessive disorders of cholesterol synthesis constitute a group of developmental malformation syndromes that cause severe effects on nervous system development and function. Mutations within cholesterol synthetic enzymes, including 3β-hydroxysterol Δ7-reductase (DHCR7), 3β-hydroxysterol Δ24-reductase (DHCR24), 3β-hydroxysteroid Δ5-desaturase (SC5D), result in Smith Lemli–Opitz syndrome (SLOS), desmosterolosis, and lathosterolosis (LATH), respectively. Though these disorders share the commonality of reduce cholesterol content, broad cellular and patient phenotypic differences of unknown origin exist between these diseases. Thus, while the associate biochemical defects are well defined, neither the specific mechanisms underlying neurological abnormalities nor the role of decreased cholesterc versus sterol precursor accumulation in disease pathogenesis have been clearly delineated.

Methods: In order to identify mechanisms underlying the neurological deficits observed in these disorders, we generated induced pluripotent stem (iPS) cells from SLOS and LATH patients containing missense or frameshift mutations within *DHCR7* or *SC5D*. These cell lines were characterized for biochemical defects by gas chromatography/mass spectrometry and assayed for neural phenotypes using molecular and genomic assays. We also utilized small molecule inhibitors of cholesterol synthesis enzymes and *Dhcr7* mouse models to analyze disease specific effects.

Results: When cultured in cholesterol deficient medias, both SLOS and LATH iPS derivatives demonstrate loss of cholesterol and accumulation of appropriate precursors, 7,8-dehydrocholesterol in SLOS and lathosterol in LATH. Neural differentiation assays revealed that SLOS iPS cells exhibit defects in progenitor maintenance and precocious neuronal differentiation, while LATH iPS cells generate and maintain neural progenitors comparable to control iPS cells. Further, inhibition of DHCR24 activity with U18666A or inhibition of HMG-CoA reductase activity with simvastatin had no effect on the differentiation of control iPS cells, further revealing differential effects of cholesterol precursor accumulation on neural specification. To identify signaling mechanisms altered in SLOS iPS cells, gene and protein expression analyses identified loss of Wnt/β-catenin signal-

Conclusions: Our work demonstrates the utility of iPS cells for modeling metabolic disorders, identifies key signaling mechanisms underlying Smith–Lemli–Opitz syndrome, and reveals phenotypic and cellular differences between cholesterol synthesis disorders.