VASCULARIZATION IN HUMAN INDUCED PLURIPOTENT STEM CELLS UNDER HYPEROSMOLARITY INDUCED BY HIGH GLUCOSE

ACC Poster Contributions
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Authors: Rosalinda Madonna, Harnath Shelat, De Caterina Raffaele, Yong-Jian Geng, Texas Heart Institute, Houston, TX, University of Texas Medical School, Houston, TX

Background: In overt diabetes, hyperglycemia (HG) increases the plasma osmolarity, leading to adaptive responses to hypertonicity stress. Cyclooxygenase (COX)-2 plays a role in angiogenesis and plaque stability. Induced pluripotent stem (iPS) cells have been used as an in vitro disease model that recapitulates the interplay between genetic and environmental risk factors in the vascular complication of diabetes. This study tested whether HG promotes angiogenesis in iPS cells through the induction of expression and activity of hypertonicity-responsive genes, including those coding for the osmosensing water channel aquaporin-1 (AQP1) and COX-2.

Methods and Results: Human iPS cells were generated from skin fibroblasts by lentiviral transduction of four reprogramming factors (Oct4, SOX2, Klf4, and c-Myc). Following 3 weeks of culture, compact refractive ES-like colonies emerged. All iPS cell colonies showed expression of OCT4, SSEA3, SSEA4 and SOX2. After reprogramming, iPS cells were characterized for their adaptive responses to HG-induced hypertonicity stress, by incubation with 5.5 mmol/L glucose (normoglycemia), high glucose (HG) at 12.5, 25 and 45 mmol/L, or with the hyperosmolar control mannitol (HM) at 12.5, 25 and 45 mmol/L for 72 hours. At the end of treatment, high-throughput screening of iPS cell number and expression of AQP1, COX-2 and Tonicity enhancer binding protein (TonEBP), were determined in cells seeded in 96 well plates. Both HG and HM decreased iPS cell numbers while increasing expression of AQP1, COX-2 and Ton. iPS cells formed bundles in methylcellulose matrix and tubing networks in matrigel, especially when they were exposed to HG and HM (HG 2.8±0.2 fold; HM 3.3±0.5 fold; n=3, p<0.01). Small interfering RNA to AQP1 reverted the inducing effects of HG and HM on COX-2 and Ton expression and angiogenic activity.

Conclusions: Under the hypertonic stimulation human iPS cells show increased expression of AQP1, COX-2 and Ton. iPS cell-derived vascular progenitor cells can form vasculatures through an AQP1-associated hyperosmolar mechanism. Targeting the osmosignaling pathway may represent a novel strategy to reduce vascular complication of diabetes.