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Notch3 is involved in adipogenesis of human adipose-derived stromal/stem cells

Demi A. Sandel, Mengcheng Liu, Ngozi Ogbonnaya, Jamie J. Newman

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Human adipose-derived stromal/stem cells (hASCs) have tremendous therapeutic potential and the ability to offer insight into human development and disease. Here we subject human ASCs to siRNA-mediated knockdown of Notch3 cultured under both self-renewing and adipogenic differentiation conditions. Self-renewal was monitored by assessing viability and proliferation rates through staining and alamarBlue assays, respectively. Adipogenesis was measured through Oil-Red O staining, western blot and quantitative real-time RT-PCR that determined expression levels of multipotency and adipogenic markers over time. Notch3 was expressed in self-renewing hASCs but knockdown, as validated by qRT-PCR and western blot, showed no impact on cell viability, as measured through live-dead staining, or cell proliferation rates, as measured through alamarBlue assays. However, as Notch3 expression was observed to increase during adipogenesis, in the absence of Notch3 there was a significant increase in hASC adipogenesis as demonstrated through an increased number of lipid vesicles, and increased expression of adipogenic markers *ppar- $\gamma$* , *adiponectin*, *fabp4*, and *plin2*. Although Notch3 is only one of four Notch receptors expressed on the surface of hASCs, this receptor appears important for proper regulation of adipogenic differentiation, possibly serving as a negative regulator to prevent inappropriate adipogenesis or promote other lineage commitments of ASCs.

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