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The Role of Notch3 in Self-Renewal of Adipose Derived Stem Cells

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Stem cells are distinguishable by their ability to 1) self-renew and 2) under certain conditions they can be induced to become specialized cells with specific functions. Their multipotency makes stem cells ideal for tissue engineering. Tissue engineering aims to regenerate damaged tissues but is an area of medicine that requires a significant amount of research and optimization before it can be widely used in the clinic. Notch signaling is a signaling pathway uniform for all metazoans and is critical in development, niche maintenance, and differentiation. In a previous study it was determined that the knockdown of Notch 3 has no effect on cell proliferation or viability. In order to validate these observations and determine if there was any impact on cell state or gene expression in the absence of Notch3, we performed RNA-Seq 72 hours after an siRNA-mediated knockdown of Notch3. The research presented here specifically investigates the effects of Notch3 knockdown on ANKRD1, NDRG2, SCRIB, IF144, CDKN3, LINC, MBTPS, and ACTC1 in human Adipose Stem Cells (hASC), genes that showed significant up or down regulation following knockdown. Primers were designed and optimized for each of these genes using end point PCR. Once optimized the primers were tested against the knockdown and control samples and results were compared with the RNA-seq data. Quantitative real-time PCR was then used to measure the effect of Notch3 knockdown. Future work will aim to further characterize the relationship between Notch3 and these affected genes to better explain mechanisms that regulate ASC self-renewal and multipotency. Understanding the cellular interactions of Notch3 will enhance future clinical applications of tissue engineering.