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Investigating Expression Levels of the Notch Pathway in Self-Renewing hASCs

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Human adipose-derived stem cells (hASCs) can be used in regenerative medicine to treat connective tissue injuries and degenerative conditions. Although hASCs provide a cell source to repair bone, muscle, and cartilage, additional studies need to be performed in order to properly characterize and manipulate these cells for use in the clinic. The Notch pathway is highly conserved across many species and consists of 4 receptors and 5 ligands, each performing vital cellular functions throughout development1,2. Here we present data on the expression of protein and transcript of genes associated with the Notch pathway in hASCs. We also decreased expression of Notch3 mediated by an siRNA to study the influence of Notch3 on self-renewal in order to better understand the dynamic nature of this pathway. Notch3 is known to specifically regulate differentiation of hASCs3, but the exact mechanism and relationship between Notch receptors during self-renewal remains poorly understood. Human ASCs were grown in complete culture media (CCM) to maintain self-renewing properties until completely confluent at which point, they were lysed to collect protein and RNA. Protein samples were collected and western blots were performed to monitor expression of Notch receptors. RNA was collected in Trizol, extracted following the manufacturers protocol, and further subjected to cDNA synthesis. Primers were designed for reverse-transcriptase polymerase chain reaction (RT-PCR) for each of the Notch receptors and ligands. All experiments compared negative control siRNA treated cells to those exposed to an siRNA mediated knockdown of Notch3. Western blot analysis was used to validate the Notch3 knockdown and demonstrated significant differences in Notch4 expression following the loss of Notch3 (Figure 1) indicating that Notch3 may play a role in regulating expression of Notch4 during hASC self-renewal. Primers have been designed and optimized to detect transcripts for notch1-4 and ligands jagged1-2, dll-1, dll-3, and dll-4, and this data will allow us to better understand the relationship between Notch3 and other members of the signaling pathway, helping to establish the mechanistic action and future manipulation of these cells for clinical application. Bibliography 1. Song BQ, Chi Y, Li X, et al. Inhibition of Notch Signaling Promotes the Adipogenic Differentiation of Mesenchymal Stem Cells Through Autophagy Activation and PTEN-PI3K/AKT/mTOR Pathway. Cell Physiol Biochem. 2015;36(5):1991-2002. 2. Hori K, Sen A, Artavanis-Tsakonas S. Notch signaling at a glance. J Cell Sci. 2013;126(10):2135-2140. 3. Sandel DA, Liu M, Ogbonnaya N, Newman JJ. Notch3 is involved in adipogenesis of human adipose-derived stromal/stem cells. Biochimie. 2018;150:31-36.