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Determining Expression Levels of the Notch Signaling Pathway in Self-Renewing hASCs

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Human adipose-derived stem cells (hASCs) have immense potential for regenerative medicine. They can potentially differentiate to form bone, muscle, or cartilage tissue making them very promising in combating degenerative diseases. One of the key elements for maximizing the clinical usage of these cells is determining mechanisms that determine cell fate. The Notch signaling pathway is highly conserved across multiple species and is made up of 4 receptors (Notch 1,2,3, and 4) and 5 ligands (Jag1, Jag2, Dll1, Dll3, and Dll4) each performing an important role in the process of cellular homeostasis and differentiation. Although this pathway is known to be significant in hASC differentiation, very little is known about the role of each receptor and how they work together to maintain and direct cell state. If hASCs are to be used in a clinical setting, then the Notch pathway must be fully understood. We investigate this pathway by determining the expression levels of each of its receptors and ligands. This allows us to piece together how the pathway works by determining how involved these proteins are in the Notch pathway. We further investigate the mechanism of this pathway by performing an siRNA mediated knock-down of Notch 3, a protein which has been determined to play a key role in the pathway. The siRNA will interrupt the translation of the Notch 3 gene from mRNA to protein which will lead to reduced expression of Notch 3. We will then look at the expression of other components of the Notch pathway. If there is a change in expression level in a protein after the Notch 3 knock-down, then there is likely a connection between that protein and Notch 3. By piecing together all of this information, we can begin to get a complete picture of the mechanisms driving the Notch signaling pathway. Once this pathway is understood, it could lead to the development of treatments for degenerative diseases using hASCs.