Notch Signaling Plays a Key Role in Regulating Adult Stem Cell Osteogenic Differentiation

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Introduction

Human adipose-derived stem cells (hASCs) are an easily accessible type of multipotent stem cell that boast immense potential as a cell source for personalized regenerative medicine. Given their tremendous clinical potential, our research strives to better understand hASCs and their potential use in combating degenerative bone diseases such as osteoporosis and trauma related degradation. We are specifically interested in deciphering the role of the highly conserved Notch signaling pathway and how it might be manipulated to enhance bone differentiation and regeneration [1, 2].

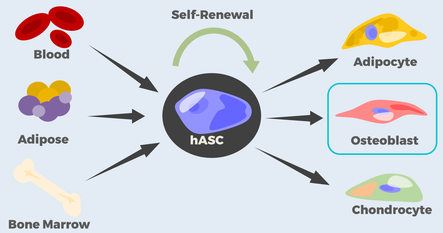


Figure 1: Human adipose-derived stem cell lineages. hASCs can differentiate into a variety of mature cell lines, including: blood, adipose, cartilage, and more. This project is specifically focusing on the pathway hASCs take to differentiate into bone cells, or osteoblasts.

The Notch signaling pathway is a cell-to-cell contact dependent pathway that is involved in cellular homeostasis, stem cell differentiation, and cell fate determination. *Notch1* and *notch3* are two receptors in the Notch signaling pathway that play a critical role in osteogenesis and self-renewal [3].

Receptors	Ligands
notch1	DLL-1
notch2	DLL-3
notch3	DLL-4
notch4	Jagged1
	Jagged2

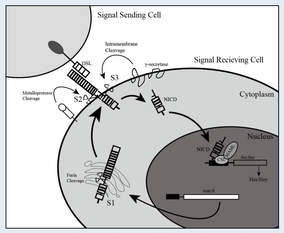


Figure 2: The Notch signaling pathway is a contact-dependent cellular pathway that is involved in regulating homeostasis, stem cell differentiation, and determining cell fate. In this project, we are focused on the *notch1* and *notch3* receptors.

Literature indicates that a decrease in *notch1* expression leads to a decrease in osteogenic differentiation. Given data in the literature for other types of cells, we expect to confirm that the decrease in *notch1* leads to an increase in hASC osteogenesis while the decrease in *notch3* may lead to decreased differentiation. We will do so by performing small-interfering RNA (siRNA) knockdowns [4].

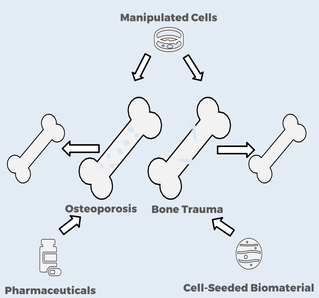


Figure 3: How different methods lead to the therapy of multiple bone-related injuries or illness.

Decreased bone density can be caused by an array of conditions including osteoporosis, bone trauma, and microgravity that astronauts experience on the ISS. Identifying the role of each receptor in the Notch signaling pathway will aid in identifying potential therapeutic targets for treating bone degeneration and loss in the future [5].

DNA

Genome Regulation

Gene expression is controlled by the environment

RNA

Transcription

Ribosome reads mRNA and builds protein

Protein

Translation

Protein is transported to different locations within the cell

Chemical Composition of Media Influences Gene Expression

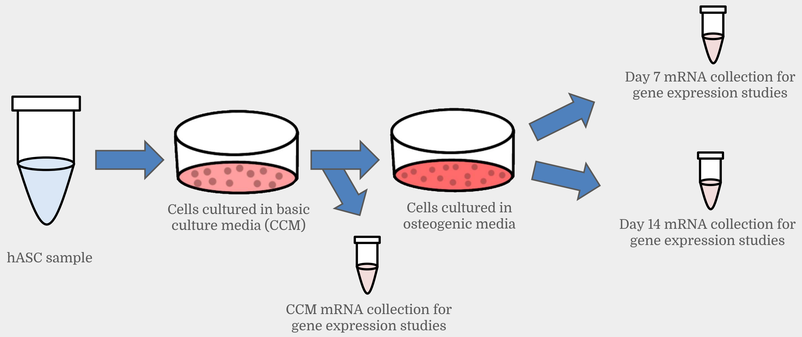
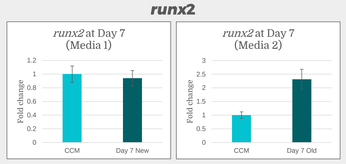


Figure 4: Cells are first cultured on basic media (CCM) before switching to osteogenic media, which then initiates differentiation. mRNA was collected at 3 different time points: Day 7, and Day 14 post differentiation. In this project, two versions of osteogenic media were used to evaluate efficiency of differentiation

Notch1 and *Notch3* Gene Expression Shifts During Osteogenesis

Figure 5: *notch1* and *notch3* gene expressions were quantitatively analyzed via qRT-PCR at both Day 7 and Day 14 with Media 1. *Notch1* gene expression levels are high at Day 7, and then dip at Day 14. Additionally, from this data, *notch3* gene expression seems to increase at Day 7, but then level off at Day 14. This is preliminary data.

Osteogenic Differentiation Determined by *runx2* Expression



The Media Dilemma

According to **Figure 6**, Media 2 yields a higher differentiation potential than Media 1. We are not sure as to why, given we do not own the recipe to Media 1. From these results, we are permanently switching to Media 2, and will continue to use it throughout the rest of our project.

Figure 6: A novel osteogenic marker, *runx2*, was quantitatively analyzed with qRT-PCR at the Day 7 timepoint with the two medias. *Runx2* gene expression was much higher in Media 2, which indicates a higher osteogenic differentiation potential in Media 2.

Alizarin Red Staining Indicates Bone Differentiation

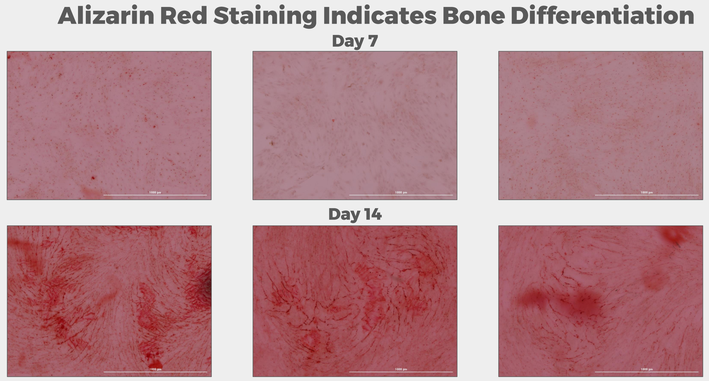


Figure 7: Alizarin Red is a dye that stains calcium nodules, which is crucial for qualitatively determining osteogenic differentiation, since bone tissue is composed of calcium. Staining is shown for two time points following induction of differentiation.

Conclusion & Future Directions

Our research is centered around understanding the role of *notch1* and *notch3* in hASC osteogenesis. Our current results suggest that:

- *Notch1* shows a decrease in mRNA transcript expression at 14 days after osteogenic differentiation has been initiated (Figure 5), suggesting a role for *notch1* earlier in osteogenic differentiation [3].
- *Notch3* shows an increase in mRNA transcript expression 7 days after osteogenic differentiation and then returns to normal expression levels suggesting that *notch3* plays a role early and throughout osteogenic differentiation[7].
- Media 2 leads to more efficient osteogenic differentiation and will be used for future studies.

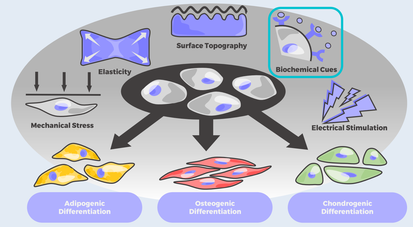


Figure 8: Human adipose-derived stem cell differentiation can be influenced by a variety of factors, most notably being biochemical cues.

Future work will strive to understand the specific role for Notch in osteogenesis. Specifically we will:

- Confirm results of gene expression through qRT-PCR for other osteogenic markers and western blots to determine protein expression of these factors.
- Perform siRNA knockdowns of each receptor while monitoring osteogenic differentiation related to these knockdowns.
- Study the effects of simulated microgravity on hASC osteogenic differentiation in the presence and absence of *notch1* and *notch3* to provide.
- Some functional information on how Notch signaling may be manipulated to help those who suffer bone loss in microgravity (Figure 9).

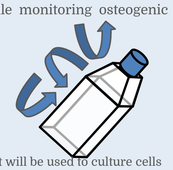


Figure 9: This is a graphic representing a tissue culture flask that will be used to culture cells in an simulated microgravity environment. The flask rotates continuously on an x, y, and z plane simultaneously while also moving in a figure-8 motion.

Acknowledgements

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Sources

[1] Frese, L., Dijkman, P. E., & Hoerstrup, S. P. (2016). Adipose Tissue-Derived Stem Cells in Regenerative Medicine. Transfusion Medicine and Hemotherapy, 43(4), 268.

[2] Zuk, P. A., et al. (2002). Human Adipose Tissue Is a Source of Multipo. Stem Cell. Mol Bio of Cell, 13(12), 4279–4295.

[3] Braune, E.-B., & Lendahl, U. (2016). Notch -- a goldilocks signaling pathway in disease and cancer therapy. Discovery Medicine, 21(115), 189–196.

[4] He, Y., & Zou, L. (2019). Notch-1 inhibition reduces proliferation and promotes osteogenic differentiation of bone marrow mesenchymal stem cells. Experimental and Therapeutic Medicine, 18(3), 1884.

[5] Sandel, D. A., Liu, M., Ogbonnaya, N., & Newman, J. J. (2018). Notch3 is involved in adipogenesis of human adipose-derived stromal/stem cells. Biochimie, 150, 31–36.

[6] Komm, B. S., Morgenstern, D., Yamamoto, L. A., & Jenkins, S. N. (2015, November 2). The safety and tolerability profile of therapies for the prevention and treatment of osteoporosis in postmenopausal women. Expert Review of Clinical Pharmacology, Vol. 8, pp. 769–784.

[7] Brack, A. S., Conboy, I. M., Conboy, M. J., Shen, J., & Rando, T. A. (2008). A Temporal Switch from Notch to Wnt Signaling in Muscle Stem Cells Is Necessary for Normal Adult Myogenesis. *Cell Stem Cell*, *2*(1), 50–59