Winter 3-2021

The Role of MED12, Notch1, and Notch3 in Adipose Stem Cell Self-Renewal and The Integrated Use of Stem Cells in Public Educational Materials

Rebecca Hodnett

THE ROLE OF MED12, NOTCH1, AND NOTCH3 IN ADIPOSE STEM CELL SELF-RENEWAL AND THE INTEGRATED USE OF STEM CELLS IN PUBLIC EDUCATIONAL MATERIALS

by

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A Thesis Presented in Partial Fulfillment
of the Requirements of the Degree
Master of Science

COLLEGE OF APPLIED AND NATURAL SCIENCES
LOUISIANA TECH UNIVERSITY

March 2021

LOUISIANA TECH UNIVERSITY

GRADUATE SCHOOL

January 14, 2021
Date of thesis defense

We hereby recommend that the thesis prepared by

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entitled **The Role of MED12, Notch1, and Notch3 in Adipose Stem Cell Self-Renewal and the Integrated Use of Stem Cells in Public Educational Materials**

Click or tap here to enter text.

be accepted in partial fulfillment of the requirements for the degree of

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ABSTRACT

Human adipose-derived mesenchymal stem cells offer significant therapeutic potential as an ethically sound, easily accessed source of adult stem cells. To harness their medically relevant properties, it is necessary to understand the mechanisms that control their fate. Cell state and differentiation of stem cells is determined by interactions of signaling pathways, chromatin modifiers, and transcription factors working to regulate cell-type specific gene expression profiles. Specifically, both the MED12 subunit of the Mediator complex and the Notch signaling pathway are known to individually influence hASC self-renewal. We investigated the relationship between Notch signaling and transcriptional cofactor, MED12, to elucidate a potential regulatory relationship and better understand the mechanisms that determine cell fate in hASCs.

Using siRNA mediated knockdowns, we analyzed the expression and activation changes of Notch signaling in self-renewing adipose stem cells in the presence of reduced MED12. Knockdown validation, Notch expression and signaling pathway activation was quantitated via qRT-PCR and western blot analysis. Subsequently, we observed that MED12 is required for the activation of the Notch3 signaling pathway, while Notch1 signaling is not significantly influenced by the reduction of MED12, suggesting a novel regulatory interface between MED12 and the Notch3 signaling pathway. Understanding the relationship between MED12, Notch1, and Notch3 and their influence on self-renewal will increase understanding of hASC cell fate mechanisms, indicating a need for further

investigation, and aiding in better determining their potential for applications in regenerative medicine.

Furthermore, to advance support for scientific investigation and essential stem cell research, public education of the basic science and medical relevance of stem cells must also be addressed. An interactive children's book was developed to integrate basic science research and stem cell concepts inside and out of formal educational facilities. Specifically designed as an educational book for students, a tool for educators, and a resource for the community, this book aims to effectively communicate fact-based stem cell content, address misconceptions, and promote positive engagement and interest in the sciences.

This thesis provides evidence of a novel regulatory relationship between Notch signaling and MED12 and contributes, via educational resource, to advancing support for essential stem cell research. Collectively, this aids in the elucidating the potential use and benefits of adipose stem cells in clinical therapeutics and regenerative medicine.

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Author Rebecca Hackett

Date January 27, 2021

DEDICATION

I dedicate this work to each member of my family for their unwavering, constant, and enthusiastic support and sacrifice through many long days and nights and difficulties faced; whom I could not have been successful without.

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ACKNOWLEDGMENTS

I would like to acknowledge with sincere gratitude, first, my advisor, Dr. Jamie Newman, and fellow members of the Newman Lab. I am ever grateful for the committed guidance, support, encouragement, and patience Dr. Newman continuously offered through the duration of the research and writing for my thesis. I am grateful for her allowing me to pursue both traditional, basic science research as well as interdisciplinary, unique aspects of science and for her believing in it, going above and beyond, and helping me see it through. Without her guidance through every step, I would not have made it through my thesis journey. A sincere thank you to Joseph Straub and Dr. Mengcheng Liu for their assistance learning techniques and answering all my many questions, Jaylen Mumphrey as a fellow graduate student on the project who was essential to its completion, as well as all other members of the Newman Lab for their comradery, help, and constant support.

I am grateful to the College of Applied and Natural Sciences for their continued support through mini grants that aided me in completing this work, as well as the Louisiana Space Grant Consortium who provided me a Graduate Research Award to fund a portion of my work on this project. Further, I would like to thank the VISTA program at Louisiana Tech along with Nick Bustamante and Dr. Jamie Newman who guided, supported, and funded the Journey of a Stem Cell. Conjointly, I would like to thank

Maddie Dearman, illustrator of The Journey of a Stem Cell, for her hard work, dedication, and inspiration that made the book come to life.

Finally, without my friends and family encouraging me every step of the way, I could not have overcome the struggles facing me to complete this thesis. To my parents for supporting me and sacrificing to do what it takes to help see me through, to my grandmother who has kept me grounded through the difficult times, cheered for me, and been my inspiration, and to my other dear friends (many of which I met along this journey) and close family that I have leaned on during this, I thank each of you for your prayers, encouragement, sacrifice, and constant support through it all.

CHAPTER 1

INTRODUCTION

1.1 Stem Cells

1.1.1 Types of Stem Cells

Stem cells hold significant potential in areas of clinical therapeutics, tissue regeneration, and disease treatment. Stem cells are cells defined by their ability to self-renew and differentiate. Their potential to specialize into multiple lineages allows them to form distinct tissues and cell types throughout the body. Collectively, there are three general types of stem cells: embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells.[1]

Embryonic Stem Cells

Previous research has identified embryonic stem cells as a pluripotent source of cells isolated from the inner cell mass of a blastocyst, lending them the ability to generate any cell type of the ectoderm, endoderm, and mesoderm germ layers. The pluripotent differentiation capacity of ESCs and determined genomic stability once had them at the center of stem cell research and regenerative medicine. Both murine and human ESCs have been investigated in depth, however, ethical issues and possible tumorigenic effects have created challenges for the further investigation and application of human ESCs in regenerative medicine, prompting the search for another stem cell source.[1]

Induced Pluripotent Stem Cells

Induced pluripotent stem cells (iPSCs) were discovered by Yamanaka, *et al.* in 2006.[2] iPSCs are generated from somatic cells by the induced overexpression of a specific set of transcription factors, which reprogram cells to a pluripotent state, restoring their self-renewing and differentiation capabilities. Though iPSCs show potency most similar to ESCs, they lack the same genomic stability, which is necessary for safe and successful application in regenerative medicine.[1]

Adult Stem Cells

Finally, adult stem cells are defined as multipotent cells with tissue-specific differentiation abilities.[1] Adult stem cells can be found in nearly every tissue in the body, existing in populations that give rise to progenitors cells: essential for tissue maintenance, cell turnover, and repair. There are many different types of adult stem cells that have been isolated from nearly all tissues. These various sources allow specific types of adult stem cells to be easily harvested with low patient morbidity, providing an ethically sound source of cells for research and clinical application (Figure 1-1).

In an effort to harness the prospective therapeutic benefits of adult stem cells, researchers have worked to improve techniques for their isolation and clonal expansion, in order to elucidate mechanisms of cell state regulation and their differentiation potential. Today, much of this research focuses on adult stem cells derived from the mesenchyme layer, known as mesenchymal stem cells (MSCs). The unique stem characteristics of these adult stem cells suggest them as ideal candidates for therapeutic and clinical utilization.

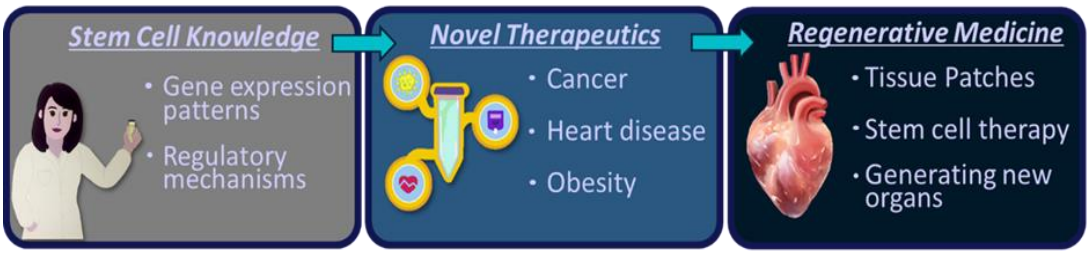


Figure 1-1: Stem cell research provides an understanding of mechanisms that control stem cell fate. The knowledge of regulatory mechanisms will lead to a greater understanding of diseases like cancer, heart disease, and obesity, as well as provide novel therapeutic targets for potential treatments. Greater knowledge of stem cell functionality may also lead to the use of stem cells in regenerative medicine. Illustration credit: Maddie Dearman.

1.1.2 Mesenchymal Stem Cells

Mesenchymal stem cells derived from somatic tissues are nonhematopoietic stromal cells,[1,3] stromal cell referring to their plastic adherent abilities, immunophenotype, and differentiation potential as determined by the International Society for Cell Therapy (ISCT).[4] MSCs are characterized by their ability to differentiate down the mesoderm lineage, giving rise to adipocytes, chondrocytes, and osteocytes[1,5] (Figure 1-2). MSCs offer a self-renewing, multipotent source of cells that have proven expandable and genetically stable *in vitro,* without the ethical and safety issues faced by other stem cell sources.[1,6] Essential for use in regenerative applications, MSCs have been shown to offer reduced alloreactivity, and thus reduced donor tissue rejection, due to low expression of MHC class I molecules combined with a lack of expression of MHC class II and their co-stimulatory molecules.[1] Paralleling this, studies have shown MSCs have the ability to home and migrate to sites of injury where their expression of chemokines aids in leukocyte attraction and tissue repair.[7] This distinct ability to influence the immune system at sites of injury is suggested to provide immunomodulatory properties *in vivo,* potentially alleviating

damaging inflammation and disease[1] and contributing to tissue repair. These distinguished traits make them a focal point in stem cell research and regenerative medicine.

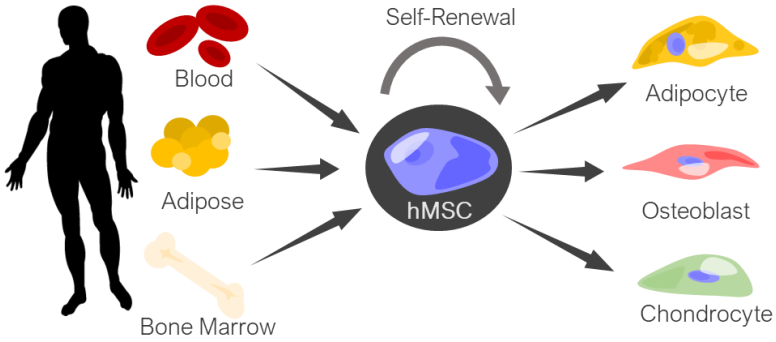


Figure 1-2: Mesenchymal stem cells can be found in many types of adult tissue including blood, bone marrow, and adipose. Human MSCs found in these tissues can self-renew and maintain a stem cell population as well as differentiate into adipocytes, osteoblasts, and chondrocytes.

1.1.3 Mesenchymal Stem Cells Derived from Bone-Marrow

Mesenchymal stem cells were first identified in bone-marrow.[1] As the long-standing primary source for MSCs, bone-marrow mesenchymal stem cells (BM-MSCs) have become the standard in stem cell therapeutics. Although mesenchymal stem cells derived from bone-marrow have been advantageous in research and instrumental in disease treatments, BM-MSCs are not the only choice of adult stem cells for clinical application. The high risk of patient morbidity from invasive harvesting practices and the cells decline in proliferation and differentiation potential with increased senescence *in vitro*,[6] has necessitated an alternative source of MSCs from somatic tissues.

1.1.4 Human Adipose-Derived Mesenchymal Stem Cells

MSCs have now been isolated from many tissues in the body. Human adipose-derived mesenchymal stem cells (hASCs) are a more recent and potentially more promising source and derivative of mesenchymal stem cells for applications in regenerative medicine. Taken from adipose (fat) tissue through a minimally invasive lipoaspiration procedure, adipose tissue contains mature adipocytes in combination with a stromal vascular fraction (SVF).[6] Fibroblasts, endothelial cells, pre-adipocytes, vascular smooth muscle cells, immune cells, and adipose stem cells are all found within the SVF,[6] in which hASCs naturally function to provide constant plasticity to replace lost and damaged tissues as part of a dynamic and highly regulated population.[8] hASCs derived from this niche are most notable for their combination of MSC characteristics, functional similarity to BM-MSCs, and their ease of access, offering low risk of patient morbidity. Existing as a multipotent population, hASCs have also demonstrated the ability to differentiate into various other cell types of the tri-germ lineage like osteocytes, neural cells, endothelial cells, cardiomyocytes, pancreatic cells, and hepatocytes *in vitro,*[6] increasing their potential in clinical applications. Various sources of MSCs show significant phenotypic and functional similarity, however, heterogeneity does exist as a result of varying tissue sources, isolation methods, and species.[7]

Human ASCs phenotypically resemble the spindly shape of a fibroblast, lacking intracellular lipid droplets[8,6] (Figure1-3). Their distinct phenotypic expression[9] includes a highly similar cell surface antigen profile when compared to mesenchymal stem cells derived from bone-marrow.[7,8] This profile, along with their complex signaling pathways and specific transcriptional mediation offer unique mechanisms of action that elevate

hASC potential in regenerative medicine. Specifically, human adipose stem cells have higher proliferation rates than that of MSCs from bone-marrow, with a lower rate of senescence and more genetic and morphological stability for long-term culture. They are characterized by immunosuppressive abilities and low immunogenicity which result from their secretion of trophic factors including cytokines, chemokines, and growth factors. Though their mechanisms of action have not been fully elucidated, hASCs show significant potential for therapeutic successes. Clinically, hASCs could address an array of concerns beyond regenerative medicine applications, as tissue-specific progenitor cells, since their benefits also include paracrine mediated signaling. This characteristic effect of hASCs influences angiogenesis, modulates inflammation, and increases cell homing to lure immune cells to sites of injury, promote cell survival, and even provide anti-scarring effects. Furthermore, there is no statistically significant correlation between hASC proliferation/differentiation capacity and patient age.[6] Altogether, these findings suggest adipose stem cells as a very promising source for regenerative medicine and stem cell clinical therapeutics.

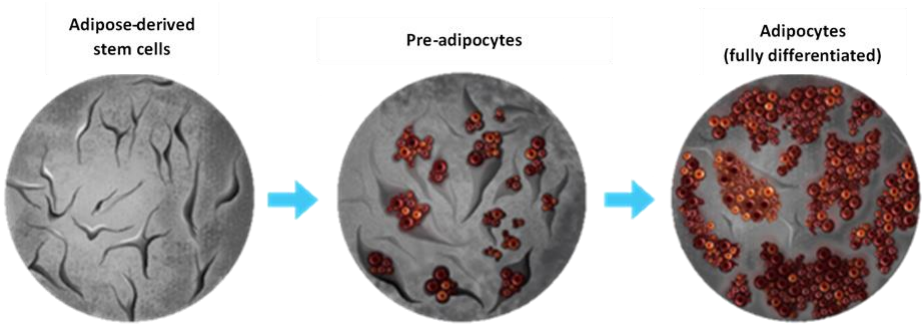


Figure 1-3: The differentiation of hASCs to lipid filled adipocytes (adipose tissue). Illustration credit: Jessica Trinh.

1.2 Cell State Regulation

1.2.1 The Role of Gene Transcription

Transcription is the fundamentally conserved mechanism by which cells express their genetic material.[5] Gene transcription in cells occurs as a response to environmental signals, queuing changes in gene expression within cells that, in turn, adapts their functionality, cell state, and cell type[10] (Figure 1-7). Because it controls nearly every characteristic and function of a cell, transcription is a highly complex and tightly regulated process where specific mechanisms of regulation work to maintain the dynamic genetic programs that are adopted based upon specific cellular requirements.[5] The high specificity of gene expression programs requires a multitude of regulatory mechanisms, all with a unique role in transcription activation and/or repression. Understanding transcriptional control in cellular mechanics is necessary for realizing the clinical potential of stem cells.[11]

Transcriptional regulation is responsible for general cellular adaptability and viability.[11] Specific to stem cells, transcriptional control is the key driving factor that determines stem cell self-renewal and differentiation.[11] Transcriptional regulation mostly involves the assembly of the pre-initiation complex and factors impacting the initiation process,[12] with protein-protein interactions integral to the process. Protein complexes consisting of interfaced transcription factors work as a "code" to regulate, recruit, and activate gene expression. [13] For transcription initiation, these transcription factors bind to target sequences at specific regulatory sites, which aids in culminating necessary transcriptional machinery to form a large, multiunit complex known as the pre-initiation complex (PIC).[5] Gene activation is dependent upon the nature of transcription factors present, as well as their spatial arrangement on the DNA.[13] Transcription factors, which

are dependent on the specific internal or external signals received from the cell, may be employed as either transcriptional activators or repressors. Activators collectively bind to enhancer regions of DNA to increase the likelihood that transcription will be initiated. The collection of these specific transcription factors on enhancer regions, in turn, recruits a molecular bridge to the pre-initiation complex to connect the transcription factors bound at the enhancer region to the basal transcriptional machinery found at the promoter. The molecular bridge, known as Mediator, is a multisubunit complex necessary for the transfer of signals from activators on enhancer regions to RNA Pol II bound at the promoter, where gene transcription will begin (Figure 1-4).[5]

1.2.2 The Mediator Complex: a Governor of Transcription

The Mediator complex was first discovered as a co-regulator of transcription in yeast and mammalian cells.[4] It has now been established as a co-activator, with a highly conserved structure and functionality that is essential for transcription.[4,14] Mediator's multisubunit structure consists of four separate modules including tail, middle, head, and kinase domains, each containing their own specific subunits (Figure 1-5). Both individually and collectively, each module has been found to uniquely contribute to Mediator's role in transcriptional regulation, though much of its function is yet to be fully elucidated.[14] The head and middle modules constitute Mediator's core domains with indispensable roles in transcribing essential protein coding genes and influencing general cell viability as they directly interact with the pre-initiation complex.[4,12] Though the tail module does contribute to Mediator's recruitment to the pre-initiation complex, via it's binding to sequence specific transcription factors, and aids in the assembly and stability of the PIC, it is not definitively required for activation, thus making it a nonessential module

for transcription. The tail, middle, and head modules of Mediator are collectively bound at MED14, which acts as the central module and backbone of the complex. Subsequently, these three modules, the tail, middle, and head, are the three modules known to be found as part of the Mediator complex when it is functionally bound within the PIC. This observation introduces the kinase domain's ability to be loosely associated with the core Mediator complex.[12] With these interactions and their resulting conformational changes, Mediator acts as a molecular bridge between the enhancer region, where activators are bound, and the core promoter, where RNA Pol II and basal transcriptional machinery provide a signal to initiate transcription (Figure 1-4).[4] Though core Mediator is thought to function in the PIC predominantly absent of the CDK8 module to initiate transcription, additional, but less pursued understandings of the CDK8-core Mediator's regulatory associations suggest a more highly complex relationship than previously understood.[15]

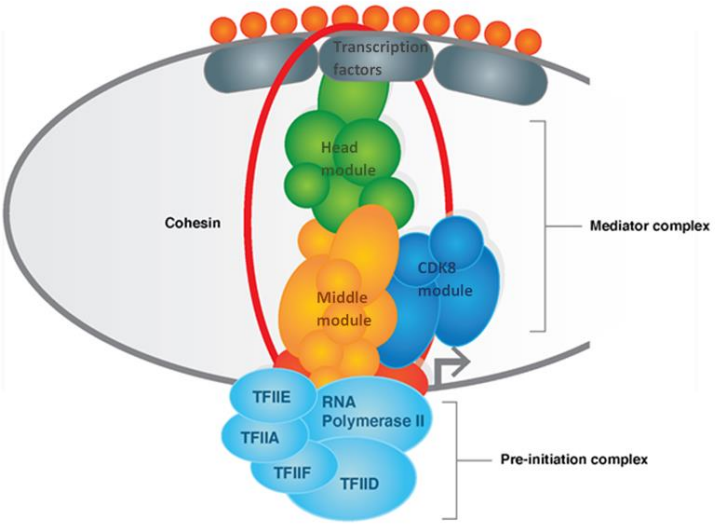


Figure 1-4: The core Mediator complex functions as a molecular bridge, transducing signals from transcription factors bound at the enhancer region to the promoter, where RNA Pol II is bound. The removal of CDK8 complex (shown here still bound to core modules) from core Mediator causes a structural change that allows Mediator to interface with RNA Poll II to initiate transcription. [15]

Protein-protein interactions that occur between the individual subunits, modules, and transcription factors influence structural adaptations to the Mediator complex.[4] Here, the subunit composition and other protein interactions govern changes that occur to the complex's structure and function.[4,13] Such interactions cause structural changes in Mediator's binding surfaces, with one of the most well characterized examples being the kinase module, which has the ability to exist autonomously from core Mediator.[11] The structural change associated with the transition from Mediator as an enhancer bound complex to a promoter bound complex is the loss of the CDK8 module. Because mediator interactions are highly dynamic and transient, the complex can contact hundreds of different transcription factors via individual interactions with various subunits.[4] Structural changes of Mediator can range from highly dynamic to slight, subtle changes, which help

to tailor interactions and determine the pool of interacting partners. This ability to interact with various partners makes Mediator highly adaptive.[11] It is also thought that because contact with the various activators is responsible for altering the structure and function of Mediator, it directly influences the signals transmitted through the complex, controlling Mediator's role in transcriptional regulation,[4] including its lesser known role in regulating various other transcriptional steps.[11] Beyond the role of Mediator to function as a co-activator, distinct roles for Mediator in transcriptional elongation, pausing, even chromatin remodeling have been suggested.[11] Mediators complex abilities in transcriptional regulation, also directly involve the specific roles of individual subunits (Figure 1-5), many of which have yet to be elucidated.[4]

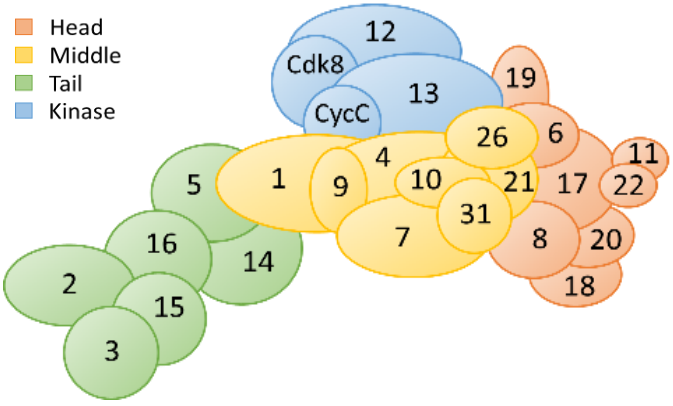


Figure 1-5: The Mediator complex consists of four separate modules: the head, middle, tail, and kinase module. Each module is made up of individual subunits, as indicated here. The core mediator module required for transcription consists of the head and middle sections. Here, Mediator is also shown bound to the tail and autonomous CDK8 module.

1.2.3 MED12 and CDK8 in Transcriptional Regulation

The kinase module consists of the cyclin dependent kinase 8 (CDK8), Cyclin C, and the MED12 and MED13 subunits. Functionally, it acts both as part of the whole

Mediator complex as well as a functional kinase autonomous from the core complex. Correlating with its own multifunctionality, CDK8 is responsible for having indirect regulatory effects on transcription through its interactions with core Mediator.[16] The CDK8 module and RNA Pol II have been shown to interact with Mediator independently, with the kinase module acting as a negative regulator. Specifically, the removal of the kinase module from Mediator is recognized as a significant regulatory step in Mediator binding to RNA Pol II in the pre-initiation complex (PIC). [14,17] If the CDK8 module is still attached, RNA Pol II is blocked from binding to Mediator, as the middle module is responsible for binding to both the basal transcriptional machinery and the kinase module.[12] Essentially, a conformational change occurs between the binding of Mediator-CDK8 and Mediator-RNA Pol II, resulting in an allosteric switch that is subcomplex dependent and responsible for RNAP Pol II activation and transcription initiation.[17]

Concurrently, autonomous CDK8 kinase activity is known to phosphorylate various transcription factors, which alters their function and marks them for degradation.[14,16] A specific example of this is CDK8's known phosphorylation of the general transcription factor TFIIH, which functions as part of the PIC, as well as it's targeting of various transcription factors in yeast cells to regulate transcription. The disassembly of the pre-initiation complex is also suggested to be a result of CDK8 kinase activity, along with the phosphorylation of RNA Pol II to disrupt Mediator-Pol II binding.[17]

Within the kinase subunit, MED12 binds directly to Cyclin C, which serves as a bridge to CDK8. Though exact mechanics are unknown, the result of MED12 binding to Cyclin C works as a switch to "turn on" CDK8 kinase activity.[18] Further, Ding *et al.* demonstrated another distinct role for MED12 where it's interaction with

methyltransferases negatively represses neuronal gene expression in non-neuronal human cells,[19] and Knuesel *et al.* suggests the ability for MED12 to have direct interactions with components in the PIC to negatively regulate transcription.[17] More recently, MED12 has been shown to regulate hematopoiesis in hematopoietic stem cells (HSCs) by regulating HSC enhancers independent of CDK8 function.[20] In this role, MED12 directly effects HSC specific gene expression programs via interaction with P3000 and colocalization with other essential hematopoietic transcription factors on active enhancers to stabilize enhancer activity and activate gene expression.[17] Collectively, the role of the Cyclin dependent kinase 8, and particularly it's subunit MED12, demonstrates significant influence on transcriptional regulation and their resulting ramifications on cell state, though many exact mechanisms are still be discovered.

1.2.4 The Indispensable Role of Notch Signaling

The Notch signaling pathway has been the topic of much research and discovery for over a century.[21] First discovered in 1917 in mutant flies and eventually characterized as an evolutionarily conserved pathway in vertebrates,[18] it is well established that Notch signaling has significant roles in developmental and regulatory functions including, proliferation, differentiation, cell death, and the activation of gene specific expression programs.[22] The complex functionality, transient nature, and context dependent specificity of the pathway, however, have made it challenging to elucidate the full scale of cellular effects and regulatory mechanisms involved. Thus far, the canonical Notch pathway has been largely established as the core Notch signal transduction pathway and is therefore thought to be involved in most Notch-dependent processes.[19]

Canonical Notch Signaling

The canonical Notch pathway (Figure 1-6) is defined by the specific sequence of proteolytic events that activate gene expression via release of the Notch intracellular domain.[19] Full length Notch proteins are transmembrane receptors found on the surface of a signal receiving cell. Their heterodimeric structure provides an extracellular region that binds to the ligand of an adjacent cell and an intracellular region (referred to as the NICD) that serves as a signal transducer, which begins a cascade of interactions that directly control gene expression.[23] Several ligands have been found to activate the canonical Notch pathway including the Jagged/Serrate proteins and the Delta-like ligand (Dll) family, which bind to the four Notch receptors found in mice and humans (Notch1, Notch2, Notch3, and Notch4).[23,21] Upon ligand binding, a conformational change combined with a mechanical pulling force from the endocytosis of the ligand, exposes a locus deep within the negative regulatory region. A metalloprotease complex, known as ADAM, cleaves the ectodomain at this site, leaving the intracellular domain attached to a small transmembrane portion. A second cleavage by γ secretase then is able to release the intracellular domain from the membrane, where it is free to translocate to the nucleus.[19] The process by which canonical Notch and its core members interact to initiate the transcription of specific target genes has been widely accepted. Once in the nucleus, the canonical Notch intracellular domain binds with a CCAAT binding protein, called CSL, in preparation to bind target genes.[22,24] This protein-protein interaction results in a molecular and functional switch of CSL from its pre-bound repressor form to a transcriptional activator, which will guide the NICD to the necessary target genes.[19] This complex further promotes the binding of RBPjk to dissociate any nearby repressors,[19] meanwhile bound by the transcriptional co-activator known as

mastermind (MAM).[19,24] Collectively, the CSL/NICD/MAM complex can then aid in recruiting necessary machinery (such as the Mediator complex) to the DNA to initiate gene transcription.[22]

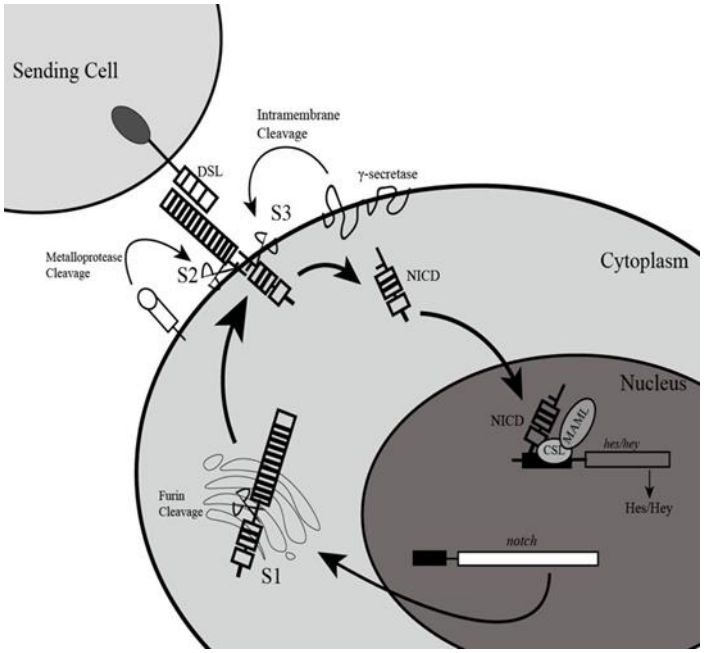


Figure 1-6: The canonical Notch signaling pathway involves a signal sending cell and a signal receiving cell. After furin cleavage and golgi processing, the full-length Notch protein is sent to the membrane as a transmembrane receptor. When a DSL ligand binds to a Notch receptor, the receptor is cleaved by enzymes in two places, releasing an intracellular domain (NICD). The Notch ICD travels into the nucleus where it complexes with transcription factors CSL and MAML to find and initiate transcription on Notch target genes.

Non-Canonical Notch Signaling

Non-canonical pathways resulting in Notch target gene activation are much less clear yet have been a significant topic of research in recent years. The non-canonical Notch pathway describes mechanisms of Notch activation or Notch protein regulatory functions derived in a ligand/CSL-independent manner. It is important to note that most of the non-canonical functions of Notch have been uncovered in stem, progenitor, or embryonic cells.

The discoveries have been largely related to the known roles of both Notch and Wnt signaling in cell fate and development, as demonstrated by evidence of Notch crosstalk with other key signaling pathways.[25] It has been suggested that because specific amino acid sequences that modulate Notch protein-protein interactions are conserved across the four Notch paralogs, specific interactions between Notch, the NICD, and/or certain factors from other regulatory signaling pathways are responsible for the complex regulation and various non-canonical effects of the pathway.[22] Thus far, influences of non-canonical Notch have been discovered in the expression of pre-synaptic vesicle proteins in neuronal cells via cell autonomous, γ secretase-independent Notch signaling[26] along with the inhibition of myoblast differentiation independently mediated from Notch1-CSL binding or the expression of known Notch targets—unlike the conventional canonical pathway.[25] Reports of non-canonical Notch interactions with the PI3K/AKT pathway, HIF1-a transcription factor, and the mTORC2 protein complex describe potential governing functions of critical cellular activities like cell migration, metabolism, survival, and differentiation.[27] Furthermore, the Delta-like 1 homolog ligands, DLK1, have been shown to directly interact with Notch1 in mammalian cells despite their absence of the DSL domain which is required for Notch-ligand interaction in the canonical pathway, demonstrating the potential influence of non-canonical ligands on Notch signaling.[28] Taken together, this suggests that mechanisms of non-canonical Notch hold key functional and regulatory roles in the decisions of undifferentiated cell populations.[25] Many of the specific mechanisms and interactions responsible for the effects of non-canonical Notch are yet to be elucidated, leaving much still to be discovered about Notch functionality.

Notch Signaling and Cell State

Specific to the potential in clinical therapeutics, Notch signaling is known to hold key influence over the self-renewal and differentiation of mesenchymal stem cells.[29,30] For example, increases in NICD1 activation have been shown to directly increase expression of canonical Notch target, Hey1, leading to elevated adipogenic and osteogenic differentiation potential in MSCs derived from bone-marrow.[30] Conversely, Notch 3 pathway activation is thought to function as a negative regulator of adipogenic differentiation in human adipose-derived stem cells, with increased Notch 3 activation inhibiting differentiation and promoting self-renewal *in vitro*.[29] More recently, it has been suggested that both Notch 1 and Notch 3 have individualized roles in hASC differentiation. Specifically, Notch 3 appears to have a critical role in maintaining stem cell state in a non-canonical manner.[31] Further adding to the importance of elucidating Notch signaling mechanisms for clinical applications is the consequence of deregulated Notch, which has been found to be responsible for an array of diseases and health disorders. Mutations in the JAG1 ligand as well as the Notch 2 receptor that result in protein truncation, are known to cause disease and functional/developmental complications of the liver and heart. Notch1 receptor mutations are associated with significant structural and developmental defects of the aortic valve, with various other genetic Notch mutations responsible for vertebral abnormalities during development. Diseases also result from the indirect influence of Notch genes. The Notch genes themselves are not mutated, but mis-regulation of the Notch pathway results from mutations of other core components of the Notch pathway. This occurs as a result of the mis-regulated pathway causing consequential increases or

decreases in the activation of the Notch pathway, their target genes, and the downstream cellular and molecular effects. Resulting health issues include chronic obstructive pulmonary disorder (COPD), pulmonary hypertension, Duchenne muscular dystrophy (DMD), amongst various other bone and cardiac diseases.[32] Additionally, dysregulated Notch signaling has been an important topic in cancer research and treatment development including various types of leukemias, breast cancers, lung cancers, intestinal cancers, amongst others. Altogether, this demonstrates the importance of fully elucidating the functional and regulatory mechanisms by which both canonical and non-canonical Notch operates for better understanding and therapeutic application.

1.2.5 MED12 and Notch Signaling Together Influence Cell State

When assessing influential mechanisms of cell function, especially with regards to stem cell therapeutics, it is reasonable to focus on the governing elements of the Notch pathway and the Mediator complex individually. However, given the overall influence of the Notch pathway on transcription and cell fate and the transcriptional control maintained by the Mediator complex in nearly all cell types, there is significant potential for interaction between the Notch signaling proteins and the subunit components of the Mediator complex (Figure 1-7) that lends a specific function in stem cell self-renewal and differentiation. Early studies in embryonic stem cells have suggested that MED12 is involved in cell fate decisions via interactions with Wnt and Notch-Delta signaling, as genetic mutations of MED12 abolished *Notch1* expression in these cases.[33] Then, in 2014, Li *et al.* discovered that the loss of Cyclin C in mouse hematopoietic cells lead to increases in Notch 1 signaling and promoted the development of T-cell acute lymphoblastic leukemia (T-ALL). This appears to be caused by the absence of active Cyclin C associated kinases, which mark the

Notch1 intracellular domain for degradation as part of the rate-limiting repression of Notch1 signaling.[34] This mechanism has since been further elucidated to show that the kinase activity of the CDK8 subunit, specifically, is responsible for targeting and phosphorylating NICD1 at the PEST domain. Because it is now known that MED12 directly influences the activation of CDK8, MED12 mutations are also known to directly influence the dysregulation of the Notch1 pathway. Without MED12-activated CDK8 kinase function to tag Notch1 for degradation, Notch 1 signaling is increased, promoting the occurrence of chronic lymphocytic leukemia (CLL).[35] Considering this, evidence is

highly supportive of potential, specific regulatory interactions of MED12 and the Notch1 and/or the Notch3 signaling pathways.

Possible applications for human adipose-derived stem cells in regenerative medicine—the ability to take one's own stem cells and employ them to regenerate lost or damaged tissues—could provide low patient morbidity, immunosuppressive effects, and increased potential for a variety of injuries and diseases that are presently limited. Today, this serves as a lofty goal in clinical therapeutics, which highlights the need for research that elucidates specific mechanisms regulating stem cell characteristics. By elucidating the

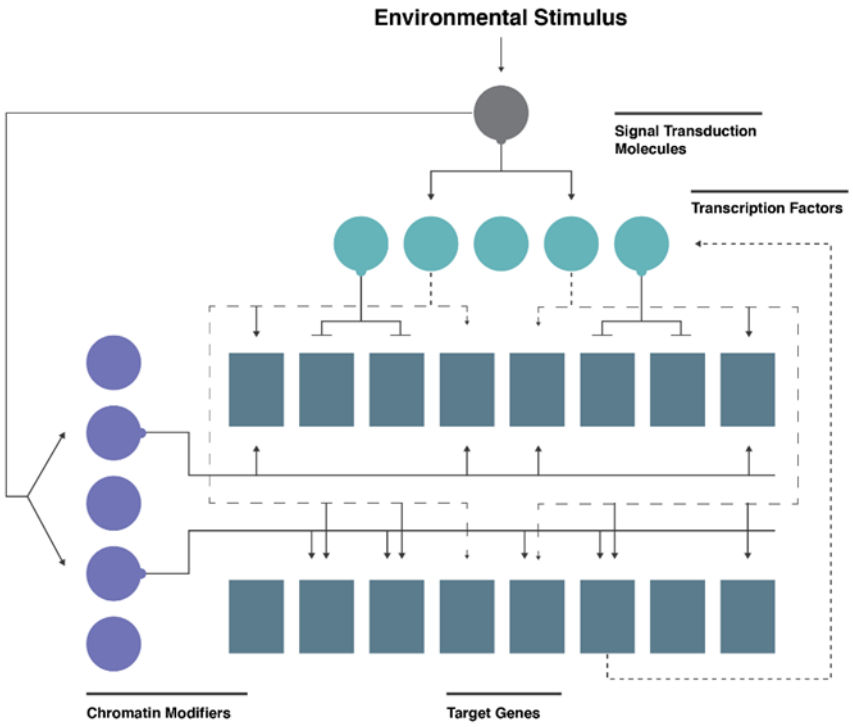


Figure 1-7: Transcriptional regulation is a highly complex process, typically initiated by an environmental stimulus. An incoming environmental stimulus activates signal transduction molecules (Notch pathway activation) which interact with transcription factors and chromatin modifiers, which interact with DNA and the pre-initiation complex (where Mediator interacts) to activate or repress the transcription of specific target genes.

potential influence of Notch1, Notch3, and MED12 interactions on hASC cell state, our insight for the optimal use of hASCs in clinical applications and stem cell therapeutics is realized. At present, despite significant evidence for the individual regulatory roles of MED12 in the Mediator complex and the Notch signaling pathway, little has been elucidated of their possible concurrent roles. Very little is known about the potential, specific interface, or relationship, between Notch1, Notch3, and MED12 in human adipose-derived mesenchymal stem cells; their specific interactions are a novel, yet promising delineation in transcriptional regulatory research. In effort to aid the understanding of possible regulatory relationships in hASCs, we determined potential relationships between MED12, Notch1, and Notch3 in regulating the self-renewing ability of hASCs *in vitro*. By analyzing changes in gene expression and receptor activation in the Notch1 and Notch3 signaling pathways after the loss of MED12 expression in hASCs, we determined MED12 plays a regulatory role in the expression and activation of Notch3. The evidence of MED12's role in Notch3 activation is a notable finding for linking MED12 to a novel regulatory mechanism for the pathway controlling the maintenance of self-renewal in hASCs.

CHAPTER 2

THE RELATIONSHIP BETWEEN NOTCH SIGNALING AND THE MEDIATOR SUBUNIT MED12

2.1 Introduction

Previous research has established significant roles for both Notch signaling and the MED12 subunit of the Mediator complex in governing specific aspects of cell state in adult stem cells. Particularly, it has been demonstrated that both the Notch1 and Notch3 signaling pathways have distinct roles in hASC maintenance and differentiation. Sandel *et al.* first identified that Notch3 expression significantly increased for 14 days after inducing adipogenic differentiation in hASCs, returning to lower levels after 21 days. They then demonstrated an increase in adipogenesis after performing an siRNA mediated knockdown of Notch3 in the cells, indicating a role for Notch3 in the regulation of hASC differentiation.[29] Lui *et al.* further elucidated unique roles for both Notch1 and Notch3 in hASC cell fate by showing that Notch3 rapidly increased 72 hours after differentiation, while Notch1 did not drastically increase until five days after adipogenesis was induced. Furthermore, their data indicated that the initial increase in Notch3 did not directly affect the expression of Notch1, supporting the idea that each receptor has a unique role in regulating adipogenesis. In addition, this observation lead to the hypothesis that the initial increase in expression of Notch3 aids in maintaining the primed stem cell self-renewing state.[36] Given these unique roles and the varied expression throughout the differentiation

process, there are likely distinct mechanisms of regulation for Notch1 and Notch3 in directing hASC cell fate.

Given the lack of conclusive data for the role of Notch1 and Notch3 in determining stem cell fate, researchers have begun to look at their intracellular interactions. Elucidating these relationships and their effects on stem cell self-renewal and differentiation will provide insight into how individual Notch receptors govern cell state. For example, recent evidence from chronic lymphocytic leukemia patients suggests MED12 has a direct influence on the degradation, and thus regulation, of activated Notch intracellular domains. Here, MED12 is responsible for activating the CDK8 kinase subunit, which in turn phosphorylates the Notch1 intracellular domain (NICD1), tagging it for degradation. This "turns off" or prevents further transcription of the NICD1 target genes, negatively regulating gene expression and the resulting effects on cell state. Without MED12, the ramifications of increased NICD1 signaling dysregulate cell state, resulting in cancerous phenotypes.[35] Considering this, and the known role for MED12 in controlling hematopoietic stem cell fate, it is likely that MED12 may regulate the Notch signaling pathway and regulate the cell fate of human adipose derived mesenchymal stem cells. By analyzing changes in *med12* and *notch* gene expression and alterations in the activation of Notch signaling pathway, this project sought to determine the relationship between MED12, Notch1 and Notch3 expression and activity in self-renewing hASCs. Here, we provide evidence of MED12 specific regulation of the Notch3 pathway via reduced pathway activation, while no regulatory for Notch1 is indicated during hASC self-renewal.

2.2 Methods

2.2.1 Cell Culture

Human adipose-derived mesenchymal stem cells (AA20181218) purchased from Obtala, Inc. were expanded in complete culture media (CCM), containing 81% MEM alpha (Gibco; 12561-049), 16% Fetal Bovine Serum (ATLANTA biologicals; S11150), 1% L-Glutamine (Gibco; 25030-081), and 1% Penicillin Streptomycin (Gibco; 25030-081). Cells were maintained in a humidified incubator at 37°C with 5% CO_2. Cells were passaged at 70%-80% confluency and viability was assessed using Trypan Blue staining and counted via Countess II FL Automated Cell Counter. Cells were seeded at 100,000 cells in 10 cm tissue culture treated plates and 45,000 cells in 6cm tissue culture plates, with media changed every 48 hours. All experiments were performed using cells passaged no more than five times to ensure multipotency and robust self-renewal.

2.2.2 Knockdown Studies

Sequence specific small interfering RNA (siRNA) were used to reduce MED12 (4392420, Thermo Fisher Scientific), Notch1 (s453558, Thermo Fisher Scientific), and Notch3(s9641, Thermo Fisher Scientific) expression. Non-specific, scrambled siRNA (AM4611, Thermo Fisher Scientific) was used to establish control groups. Cells were transfected at 50% confluency using 10nM siRNA, RNAi Max Lipofectamine (13778075, Thermo Fisher Scientific), and Opti MEM (31985062, Thermo Fisher Scientific). Media containing siRNA and transfection reagents was replaced with CCM 24 hours after siRNA treatment.

2.2.3 RNA Extraction, cDNA Synthesis, qRT-PCR

Total RNA extractions were performed using TRIzol reagent (15596018, Thermo Fisher Scientific) and chloroform, following the manufacturer's protocol and quantified based on absorbance at 260nm. Using 1,000ng of RNA, cDNA was synthesized via qScript cDNA SuperMix (101414106, Quanta Biosciences). Quantitative reverse transcription PCR (qRT-PCR) was performed to quantify transcript expression levels for *MED12, Notch1, Notch3* (Table 2-1) using PowerUp SYBR Green Master Mix (A25742, Thermo Fisher Scientific), as designated by the manufacturer's protocol, on a StepOnePlus real-time PCR system (Thermo Fisher Scientific). Transcription levels were normalized to GAPDH and fold change was calculated via the ΔΔCt method. Biological triplicates with technical replicates were used for each data set, with data reported as the average of the biological replicates with error bars denoting the stand error of the mean (SEM).

Table 2-1: Sequences of Primers

Gene name	**Forward Sequence (5' to 3')**	**Reverse Sequence (5' to 3')**	**Product size (bps)**
gapdh	ACTAGGCGCTCACTGTTCTCT	CAATACGACCAAATCCGTTGACT	99
med12	CGAAAAGGGACAGCAGAAAC	CCCATCCTCCCCACCTAAGA	87
notch1	CACGCTGACGGAGTACAAGT	GGCACGATTTCCCTGACCA	56
notch3	CACCCTTACCTGACCCCATCC	TTCGGACCAGTCTGAGAGGGA	81

2.2.4 Protein Extraction and Western Blot

Cells were washed twice with PBS and collected on ice. Total protein was extracted using Pierce RIPA buffer (89900, Thermo Fisher Scientific) with Halt Protease & Phosphatase Inhibitor Cocktail (78441, Thermo Fisher Scientific) added. Cell lysates were

rotated for 30 minutes at 4°C, then centrifuged for 20 minutes at 12,000rpm at 4°C. Collected protein was quantified via standard curves generated from BSA based Bradford assays (5000006, Bio-Rad). Laemmli sample buffer (1610737, Bio-Rad) and heating were used to denature proteins in preparation for electrophoresis. Standard protocol SDS-PAGE was performed using Mini-PROTEAN TGX Gels (4561084, Bio-Rad) to separate proteins via electrophoresis. Proteins were transferred to a PVDF membrane (88518, Thermo Fisher Scientific), then incubated with blocking buffer consisting of 5% non-fat milk (9999s, Cell Signaling Technology) and TBST (Tris Buffered Saline with 0.1% Tween 20) at room temperature for two hours. Membranes were cut into designated pieces and primary, target specific antibodies (Table 2-2) were added to the blocking buffer, accordingly, and incubated overnight at 4°C. Probed blots were rinsed with TBST 3 times at 5 minutes each and incubated with the corresponding secondary antibody and Precision Protein StrepTactin-HRP Conjugate (1610308, Bio-Rad). Blots were developed and imaged via Clarity Western ECL Substrate (10705060, Bio-Rad) and Chemi Luminescence. ImageJ analysis was used to quantify protein targets, with each target normalized to GAPDH.

Table 2-2: List of Antibodies used in Western Blots

Primary Antibody	**Catalog #**	**Vendor**	**Dilution**
GAPDH	Ab9485	Abcam	1:3000
MED12	A300-774A	Bethyl	1:1000
Notch1 (C-20)	Sc-6014R	Santa Cruz Biotechnology	1:1000
Notch1	20687-1-AP	Proteintech	1:1000
Notch3	2889	Cell Signaling Technology	1:1000

2.2.5 Statistical Analysis

Statistical analysis was performed on Western blot data using a student's two tailed t-test to establish statistical significance.

2.3 Results

2.3.1 MED12 and Notch1 Likely Do Not Work Together to Regulate hASC Self-Renewal

Considering the evidence of Notch1's unique, yet separate role in regulating hASC cell state[36] along with previous evidence of MED12 directly influencing the Notch1 signaling pathway in lymphocytic leukemia cells,[35] we determined it valuable to investigate possible interactions between Notch1 and MED12 in effort to better clarify regulatory mechanisms governing the influence of Notch signaling in hASC cell state determination. To elucidate possible influences of MED12 on Notch1 signaling, hASCs transfected with MED12 siRNA were used to analyze changes in expression and signaling activation of Notch1, in the presence of reduced MED12. Triplicates of RNA and protein samples were collected 72 hours after transfection. Preliminary semiquantitative PCR data suggested possible reduction in Notch1 transcript, therefore, qRT-PCR was used to validate sufficient reduction of MED12 expression after knockdown (Figure 2-1A). Quantitative RT-PCR also demonstrated a slight reduction in Notch1 transcript 72 hours after MED12 knockdown (Figure 2-1A).

To further examine the possibility of MED12 regulation in the Notch1 signaling pathway, Western blot analysis was used to evaluate both the full length transmembrane Notch1 receptor and the activated Notch1 intracellular domain in the presence of reduced

MED12. Approximately a 70% drop in MED12 protein was quantified via ImageJ to validate successful MED12 protein reduction (Figure 2-1B). Despite demonstration of successful MED12 protein reduction (Figure 2-1B) and slight reduction in Notch1 transcript after MED12 knockdown (Figure 2-1A), we did not observe significant changes in either the full length Notch1 transmembrane receptor or the activated Notch1 intracellular domain (Figure 2-2A).

Given recent evidence from other studies in the lab indicating low levels of Notch1 expression during, we also wanted to evaluate whether or not Notch1 is expressed at high enough levels in self-renewing hASCs to have an essential role in influencing hASC self - renewal. To establish this possibility, triplicate samples of self-renewing hASCs were cultured and harvested without performing a knockdown to determine general expression of Notch1 in untreated self-renewing hASCs. Western blot was performed for the activated Notch1 intracellular domain to analyze Notch1 signaling activity in the untreated, self-renewing cells, but the NICD1 protein bands remained undetectable (Figure 2-2B). Given this, we suspect Notch1 signaling is not consequentially active during hASC self-renewal.

Lastly, to ensure full elucidation of Notch1-MED12 regulatory mechanisms in self-renewing hASCs, a Notch1 knockdown was performed. By reducing Notch1 expression, we were able to analyze any possible effect reduced Notch1 may have on the expression of MED12. Quantitative RT-PCR was used to both validate Notch1 reduction and analyze the transcript levels of MED12 after Notch1 knockdown (Figure2-2C). The expression of MED12 transcript was not significantly influenced by the reduction of Notch1, 72 hours after transfection in self-renewing hASCs (Figure 2-2C).

Collectively, though Notch1 transcript appears to be slightly reduced after MED12 knockdown, this data suggests that MED12 does not have a role in the regulation of Notch1 signaling. Given the general lack of Notch1 expression in hASC self-renewal in combination with the observed lack of MED12 influence on Notch1 expression and signaling activation, it is unlikely MED12-Notch1 significantly affects hASC self-renewal. Furthermore, given this data supports previous findings of low Notch1 expression in self-renewing hASCs, and no obvious effect on Notch1 signaling activation was observed after MED12 knockdown, we opted not to pursue further validation of low Notch1 expression/activation levels in self-renewing cells and its relationship with MED12. Instead, the focus of the project quickly turned to Notch3.

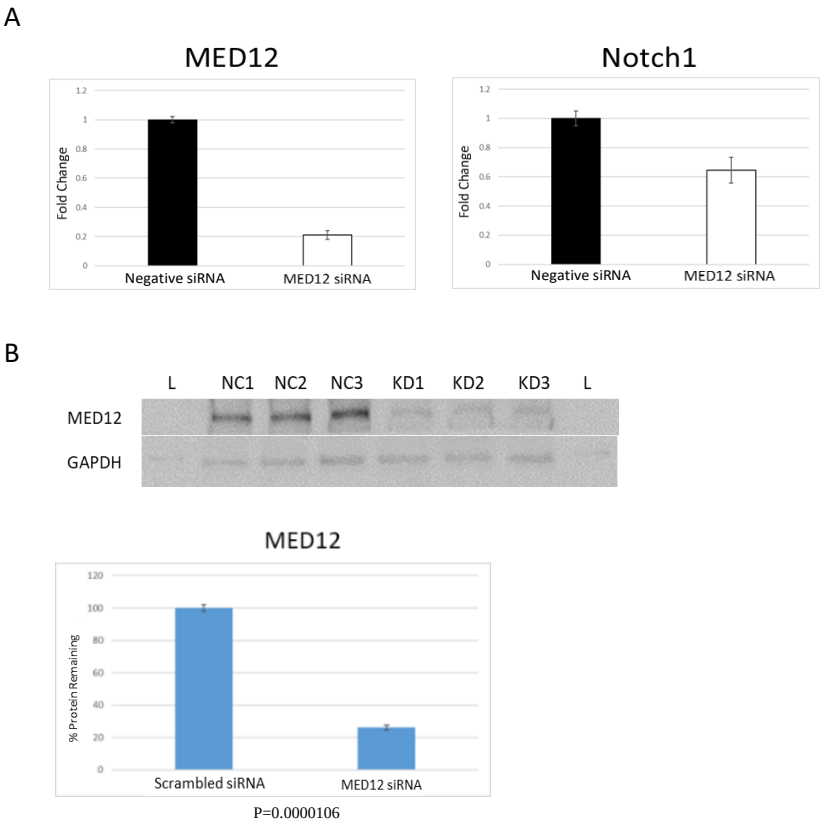


Figure 2-1: Validation of MED12 knockdown. **A.** MED12 expression was quantified 72 hours after transfection to demonstrate successful knockdown via quantitative RT-PCR. Quantitative PCR analysis also demonstrated a slight drop in Notch1 transcript expression in the presence of reduced MED12. Data represents averages of biological triplicate samples, normalized to GAPDH. **B.** Western blot was performed to validate successful reduction in MED12 protein after knockdown. ImageJ analysis was used to quantify precent protein remaining after knockdown. Western blot analysis represents biological triplicates of whole cell lysates, normalized to GAPDH**.**

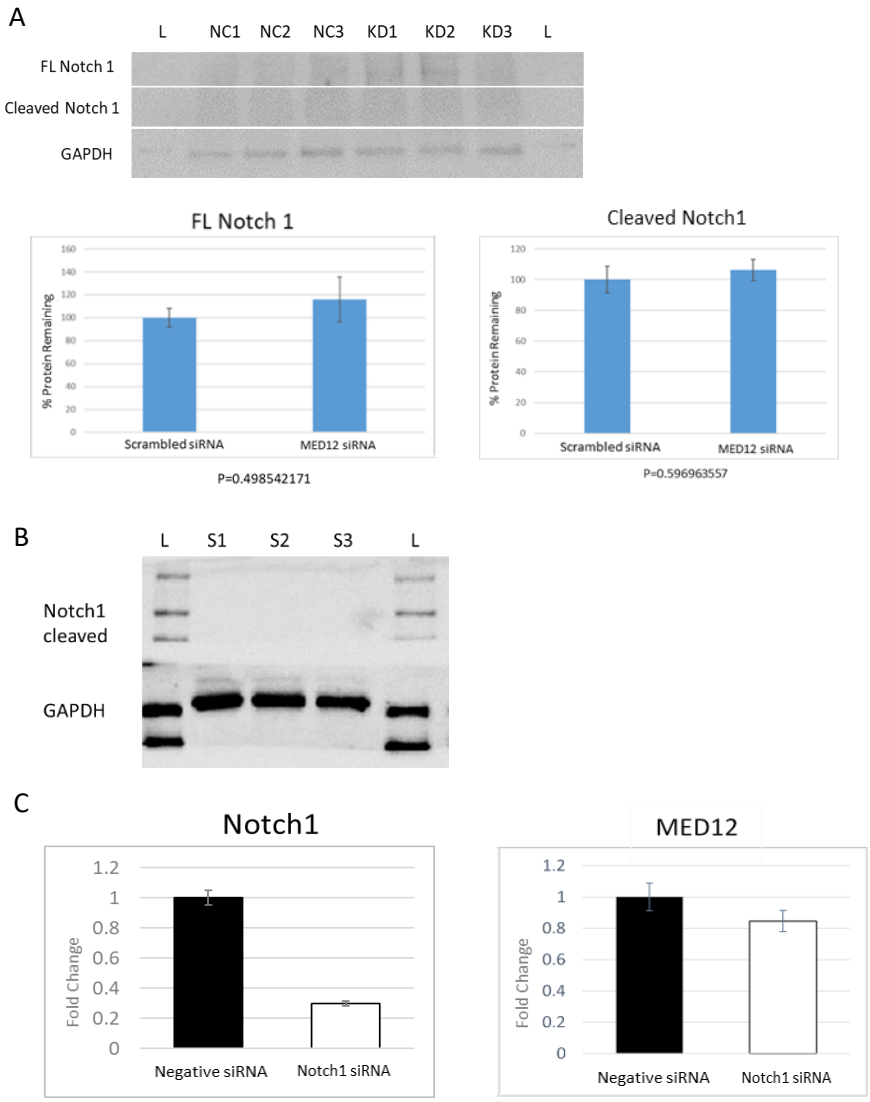


Figure 2-2: MED12 knockdown does not significantly affect the activity of Notch1, 72 hours after transfection. **A.** Western blot and protein quantification via ImageJ analysis demonstrates no changes in Notch1 proteins, 72 hours after MED12 knockdown. Full length Notch1 transmembrane receptor shows no large increases or decreases in the presence of reduced MED12. The activated Notch1 domain also demonstrated no significant change and does not appear to be present in neither the NC nor KD samples. Biological triplicates of whole cell lysates are represented, normalized to GAPDH. **B.** Western blot analysis of the general expression of activated Notch1 intracellular domain in biological triplicate self-renewing hASC samples. No active Notch1 signaling is observed in general expression samples. Western blot performed by Jaylen Mumphrey. **C.** Notch1 knockdown does not significantly affect the expression of MED12 transcript. Quantitative RT-PCR demonstrates a reduction in Notch1 expression 72 hours after Notch1 knockdown. No change is observed in MED12 expression in the presence of reduced Notch1. Data sets represent the averages of biological triplicate samples, normalized to GAPDH.

2.3.2 MED12 Plays a Role in Regulating Notch3 Signaling Activation

To determine the potential regulatory role of MED12 on the expression of *notch3* in hASC self-renewal, hASCs were transfected with sequence specific MED12 siRNA. 72 hours after transfection, biological triplicates of RNA were collected. Preliminary assays using semiquantitative RT-PCR suggested successful knockdown and differential expression of Notch3 in cells transfected with the MED12 specific siRNA. Efficiency of the knockdown was first confirmed by measuring both transcript (Figure 2-3A) and protein expression (Figure 2-3B) of MED12, where the qRT-PCR and ImageJ analysis demonstrate and approximately 70%-80% reduction in transcript and protein, respectively, after knockdown.

Quantitative RT-PCR was used to quantify the differential expression of Notch3 after MED12 knockdown. Expression analysis demonstrates a significant reduction in Notch3 mRNA 72 hours after MED12 was reduced (Figure 2-3A). This suggests that MED12 may have a regulatory effect on the expression of *Notch3*.

To understand the possible effects of MED12 on the activity of the Notch3 signaling pathway, Notch3 protein levels were analyzed via Western blot. Protein samples were collected in biological triplicates 72 hours after transfection. Western blots were performed to analyze both the full length and activated intracellular domains (NICD) of the Notch3 receptor. The full length being the inactivated, transmembrane bound receptor and the NICD being the cleaved domain of the canonical pathway. Given the decrease of Notch3 mRNA expression after MED12 knockdown, it was assumed a similar pattern would be seen with reduced protein levels in the knockdown samples. Conversely, after MED12 knockdown, we observed increases in Notch3 full length transmembrane protein,

while the activated Notch3 domain was simultaneously reduced (Figure 2-3C). Decreases in the activation of the Notch3 ICD could explain the rise in the full-length transmembrane receptors remaining in the membrane, considering that Notch3 transcript is only slightly decreased. Taken together, our observation suggests that MED12 is essential for Notch3 activation in self-renewing hASCs.

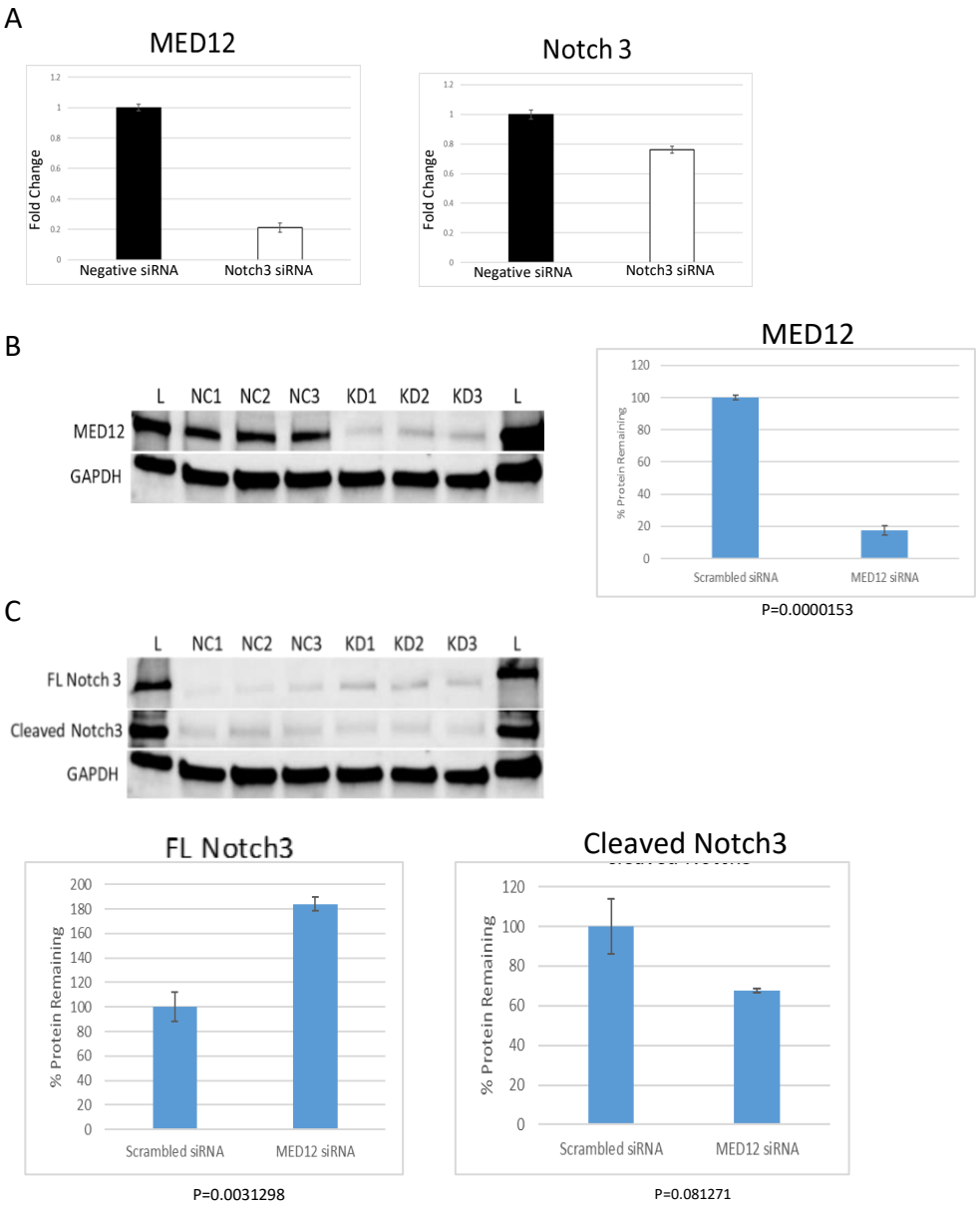


Figure 2-3: MED12 knockdown reduces Notch3 signaling activation. **A.** MED12 transcript was approximately 70% reduced 72 hours after knockdown. Expression levels of Notch3 transcript also decreased in the presence of reduced MED12, as determined by quantitative RT-PCR. Data points represent the average of biological triplicates, normalized to GAPDH. **B.** Western blot analysis demonstrates a significant drop in MED12 protein remaining 72 hours after transfection. Western blot analysis represents whole cell lysates of biological triplicates (left) and ImageJ analysis for protein quantification (right), with each sample normalized to GAPDH. **C.** Percent protein remaining of the full length Notch3 intramembrane receptor and the cleaved Notch3 intracellular domain 72 hours after MED12 knockdown. The inactive, full length Notch3 receptor presents a notable increase, while the active Notch3 domain demonstrates a similar NICD3 reduction. Western blot analysis was performed on three biological replicates with protein quantification via ImageJ. Each sample was normalized to GAPDH.

2.3.3 Notch3 Does Not Play a Role in Regulating MED12

Considering the potential regulatory role of MED12 on the Notch3 signaling pathway, it was necessary to further analyze the relationship between MED12 and Notch3 by investigating potential changes in expression of MED12, in the presence of reduced Notch3. A Notch3 knockdown was performed on triplicate cultures of self-renewing hASC via transfection with Notch3 specific siRNA. 72-hours after transfection, RNA and protein samples were collected and analyzed for changes in MED12 expression.

Preliminary transcript expression data validated the knockdown of Notch3 in the triplicate samples (Figure 2-4A). Quantitative RT-PCR also demonstrated little to no change in MED12 expression after the successful reduction of Notch3 in self-renewing cells, suggesting Notch3 has no effect on the expression of MED12 (Figure2-4A). To investigate potential regulatory protein-protein relationships of Notch3 and MED12, Western blot analysis was used to analyze changes in the protein levels of MED12 after knockdown. Despite validation of significant Notch3 protein reduction after knockdown (Figure 2-4B), MED12 does not appear to be notably changed in the knockdown samples. Quantification via ImageJ analysis further establishes a lack of change in MED12 protein levels after Notch3 knockdown (Figure 2-4C). Collectively, this suggests that the Notch3 signaling pathway, specifically the activation of NICD3 and its associated complexes, does not have a significant role in the regulation of the MED12 subunit.

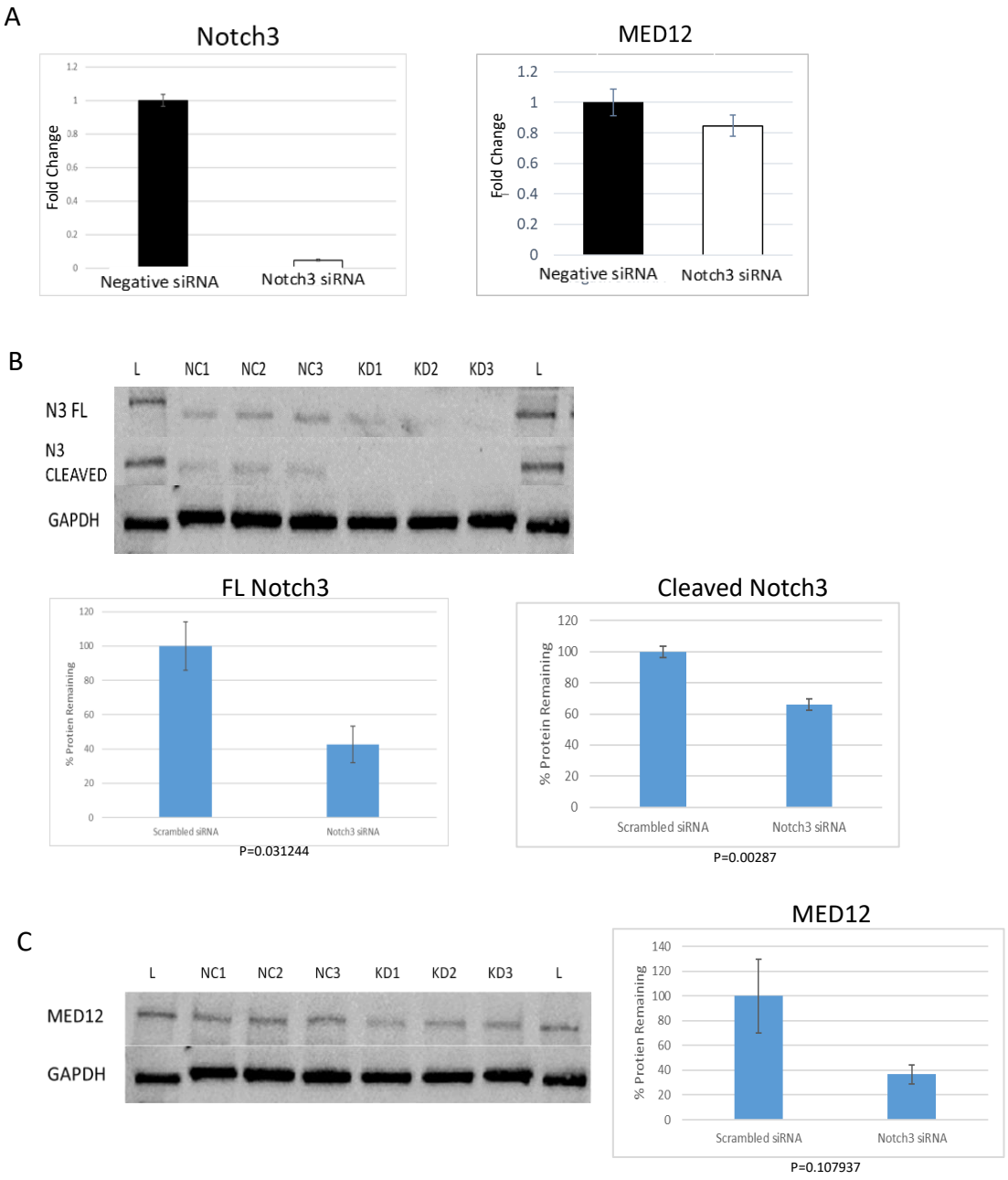


Figure 2-4: Notch3 knockdown does not significantly affect MED12. **A.** 72 hours after transfection, Notch3 transcript was reduced. qRT-PCR analysis of MED12 expression does not demonstrate a reduction in MED12 transcript 72 hours after Notch3 knockdown. Data points represent the average of biological triplicates and are normalized to GAPDH. **B.** Western blot analysis demonstrates a drop of both the full length Notch3 and activated Notch3 proteins 72 hours after transfection to validate effectiveness of the knockdown. Whole cell lysates of biological triplicates are represented via western blot, with ImageJ analysis used to quantify protein bands. Each sample was normalized to GAPDH. Western blot performed by Jaylen Mumphrey. **C.** The percent remaining of the MED12 protein after Notch3 knockdown is observed to be insignificantly affected 72 hours after Notch3 knockdown. Western blot (left) and ImageJ analysis were used to visualize and quantify protein changes, with biological triplicate samples normalized to GAPDH. Western blot performed by Jaylen Mumphrey.

2.3.4 Discussion

Cell fate requires a delicate, highly regulated balance of transcriptional control mechanisms, collectively dictating cell state and function. The previously defined roles for Notch signaling in the self-renewal and differentiation of human adipose stem cells are critical for the determination of cell state. By linking this to the evidence we provide here of a relationship between Notch3 signaling and MED12, we identify a novel mechanism for Notch3 regulation in hASC self-renewal. Using siRNA-mediated knockdown to reduce MED12 transcript and protein levels, we observe an increase in Notch3 full-length intramembrane receptors, while the cleaved, activated Notch3 domain levels simultaneously decreased in the presence of reduced MED12. Based on this data, we propose a mechanism for further investigation of the specific regulatory interactions of MED12 and Notch3 in the maintenance of hASC self-renewal. Specifically, we speculate that the increase of full length Notch3 receptor and concurrent decrease in activated Notch3 domains, is a result of regulatory interactions from MED12. In further consideration, this does not fully account for the mechanism influencing the reduction in *notch3* transcript after MED12 transfection, though it may suggest the possibility of a positive feedback loop in Notch3 signaling, given the reduced activation of the pathway paralleling reduced expression after knockdown. Thus far, specific interactions between MED12 and Notch signaling in human adipose derived mesenchymal stem cells have not been explicitly elucidated. This study suggests a novel mechanism whereby MED12 is part of regulating Notch3 signaling activation and therefore influences cell state of hASCs. These findings aid in our understanding of hASC biology and the potential role for these cells in

regenerative medicine and therapeutics, collectively proposing the need for further investigation and elucidation of intracellular interactions.

Our data determined Notch1 is likely not consequentially active during self-renewal in hASCs. In combination, we also demonstrated that MED12 likely does not significantly regulate the activity, or lack thereof, of the Notch1 pathway during hASC self-renewal. This data aligns with previous evidence and also further suggests a separate mechanism of regulation for the Notch1 and Notch3 pathways.[36] However, it is necessary to consider the decrease in Notch1 expression 72 hours after MED12 knockdown. It is possible, given its vast array of regulatory relationships and its connection to the Mediator complex, that MED12 plays a general regulatory role in the expression of both *notch1* and *notch3* transcripts, unrelated to its effect on the Notch3 pathway.

Previous studies in combination with the data presented here raise the possibility that regulation by MED12 is a result of cross-talk between various pathways or known regulatory mechanisms. Notably, recent evidence indicated that a Notch3 knockdown reduced the levels of active cytoplasmic and nuclear β-catenin in differentiating hASCs. Analysis via co-immunoprecipitation (Co-IP) of Notch3 in the differentiating hASCS revealed direct interaction of Notch3 and β-catenin, indicating that the cross-talk of the Notch3 and canonical Wnt signaling pathways could be a mechanism for the distinct regulatory role of Notch3 in hASCs.[36] When considering the impact of MED12 on the Notch3 pathway, it is also necessary to acknowledge previous evidence of MED12 in HeLa cells directly interacting with β-catenin. In this context, an interaction with MED12 is required for β-catenin to mediate transcription of Wnt-responsive genes, thus regulating β-catenin signaling and it's downstream affects.[31] Here, our data demonstrate an increase in

the amount of membrane-bound Notch3 in MED12 siRNA treated cells, in combination with decreased levels of the activated NICD3, suggesting an overall lack of signaling activation for the pathway. This lack of pathway activation in turn suggests inhibited ligand formation and/or interaction with the Notch3 receptors or the inability of receptor activation via metalloprotease and gamma secretase cleavage. The likely non-canonical relationship between Notch3 and β-catenin may also be essential for further elucidating the role of MED12 in Notch3 signaling. Given evidence for β-catenin directly inducing the expression of Jagged1 in lung cancer cells, a known ligand and activator of the Notch3 pathway,[31] the regulation of MED12 may rely on its interaction with β-catenin and the resulting downstream expression of Jagged1. Taken together, the data in this document along with recently published research suggest the need for further investigation to fully elucidate the exact mechanism of MED12 regulation on Notch3 signaling.

CHAPTER 3

THE JOURNEY OF A STEM CELL: AN EDUCATIONAL TOOL

3.1 Project Motivation

Just as important as understanding the mechanisms and techniques that make stem cells a valuable medical resource, is the ability to communicate this information to support the growth and direction of research, medicine, and healthcare in our future society. The communication of science occurs in many forms, with one of its most consequential public impacts occurring in elementary, middle, and high school classrooms.[37,38] To date, despite significant research supporting the importance of inquiry-based learning techniques in the pursuit of creating thinkers[39]—much in the way that scientists think, explore, and discover new information—there is a current overwhelming absence of applied science in these settings.[37,38] With effectively developed scientific tools and materials that demonstrate real science in various educational settings, students can be exposed to relevant experiences that develop critical learning skills.[40,41] This introduction will discuss the educational impacts created by the absence of real-life science in educational settings, the effects of deficient content knowledge of educators, and the scarcity of pertinent educational resources; together giving rise to students who are ill equipped and ill assured to pursue careers and higher education in Science, Technology, Engineering, and Math (STEM) fields.

Research in STEM education indicates that students are more successful in understanding science-based content in the classroom when exposed to direct incorporation of scientific investigation and literature.[39,42] Carefully designed, real-world science applications promote collaboration across varying levels of knowledge and experience—peers, teachers, and content professionals[43]—that ignite cognitive processes, apply concepts, and expand critical cognitive skills.[39,40] The open-ended investigations that employ critical thinking, creativity, understanding, communication, conceptualization, analysis, and questioning of a topic challenges student to achieve higher levels of understanding and apply essential information.[41] Education research investigating the integration of applied life science methods and skills has shown significant gains for students, favoring the application of investigative processes and lending increased intention to pursue higher education in science-based degree programs.[39,43] By recognizing their own ability to discover the unknown in ways that make a real and positive difference in society, students are more likely to be positively aware of the impacts of science and research and find the niche that allows them to participate in impactful science.

Unfortunately, many classroom practices employed by educators lacking appropriate levels of professional content knowledge are often unable to demonstrate relevant, real-world topics and experiences.[44,45] In turn, linear presentations of scientific content and scientific methods are depicted as a specific set of steps that implies a universal method and result.[42,46] This practice often leads to misconceptions and a limited set of critical thinking and investigative skills gained by students.[44,45] This lack of application has significant negative impacts on student learning that can result in stereotypically negative ideas of science and scientists.[46] Concurrent with the application of relevant

inquiry-based science, are the specific materials designed to implement and engage learners with the skills and techniques needed to succeed. Appropriately designed and utilized materials have significant impacts on learners, and require engaging, fact-based materials.[40] In fact, specific investigations into student reported reasons for lack of interest in scientific disciplines revealed that student responses paralleled their educator's own relationship and incorporation of content knowledge.[44,45] Furthermore, to highlight the impacts of imbedding STEM precursors in educational settings, research has shown that students with increased exposure to research-based science practices are more likely to pursue science-based careers.[43] Broadly, the inclusion of scientific investigation and literacy leads to future success in an array of professions and industries. The challenges of investigative collaboration aid student self-efficacy and improve learner outcomes in academically challenging situations.[38,47,] General familiarity with STEM topics and processes may also decrease the likelihood of disconcerted public opinions,[36,38] potentially providing more support and resources for impactful investigations in the future. When education settings are developed with relevant, applicable understanding of applied science, positive attitudes towards science lead to a greater likelihood of scientific understanding, in turn promoting generations of highly informed students, professionals, and citizens.[40,47]

In an effort to address the need for educational materials in the critical area of stem cell biology and current investigative techniques to increase public knowledge and interest, this chapter will describe the development and dissemination of an interactive children's book. By creating a book that is contextually accurate, engaging, interactive, and informative, the book communicates basic stem cell biology, relevant science, addresses

common stem cell misconceptions, and functions as an educational tool to promote interest in stem cell research and scientific investigation.

3.2 Methods

3.2.1 Content Design: Text

Components in the book described via text were intentionally developed to identify, explore, and clarify key elements of stem cells, research, and their collective medical relevance. The text was written in close collaboration with the artist to ensure that what was written targeted specific concepts and appropriately described the included illustrations. To the reader, the text is intended to communicate, in appropriate detail, a fundamental knowledge of stem cells and the "how" and "why" behind their essential role in medical research. The explanations, questions, concepts, and challenges included via text in the book allows readers to confront common misconceptions about stem cell research and develop a sense of relevancy and curiosity for how clinical applications involving stem cells could potentially impact their own lives. This appropriation of knowledge conveyed through the text is aimed at increasing reader interest and motivation in science, research, and general STEM.

Components of the book were constructed around specific organizational strategies. By chunking,[48] or presenting specific sets of information in easier to understand groups, three primary sections were developed: background, stem cells and research, and importance (Figure3-1). The background and introductory portion of the text were established to introduce the reader to basic stem cell biology, information that some readers may or may not have previous knowledge of. This text conveys background information including an overview of what stem cells are and what they do, specifically explaining their

ability to self-renew and differentiate. To establish reader knowledge of fundamental functions and characteristics of stem cells, this section addresses where and why stem cells self-renew and differentiate inside of our bodies, and why the natural abilities of our stem cells are imperative to our development and health. This portion of the book prepares the audience to address the key themes to follow and exposes them to the motivation for the book.

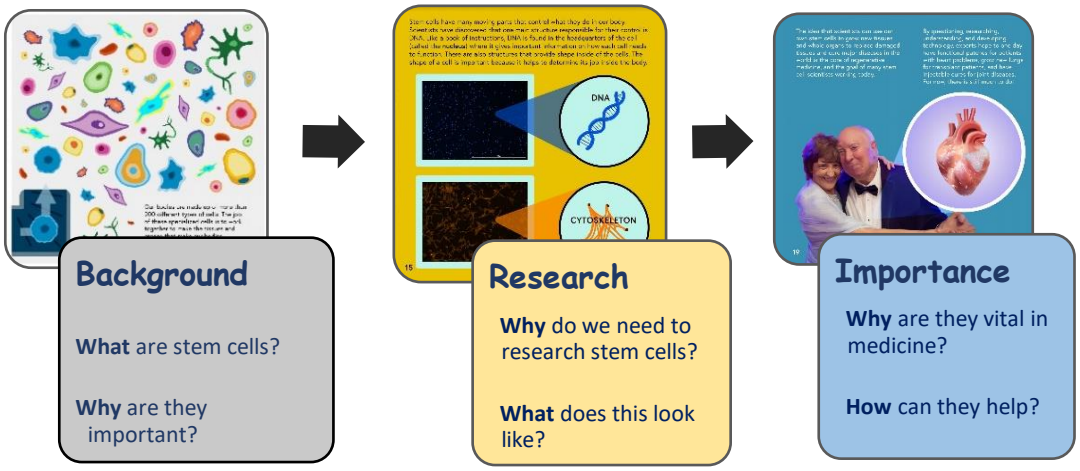


Figure 3-1: Organizational framework for the The Journey of a Stem Cell. This figure represents the informational flow of the book and outlines the general information and/or misconceptions addressed in each section. Illustration credit: Maddie Dearman.

The second segment of the book is aimed at conveying specific information related to stem cell research. The text shifts to portray the relevance of stem cells in research and elements of scientific investigation including interdisciplinary collaborations and careers that contribute to the research. Specifically, the text describes how the natural abilities of stem cells (from the previous section) can be harnessed and used in clinical applications and depicts what stem cell research looks like today. The key points presented in this text largely work towards addressing common misconceptions, disseminating knowledge of

current stem cell research practices, and peak reader interest for the potential offered in various roles and careers involved in stem cell research.

The third section of the text was developed to describe the overall significance of stem cell research by describing what scientists are investigating and how this could positively impact the treatment of many diseases and medical conditions. The concept of regenerative medicine is described here in order to communicate the future of stem cell research. This section intentionally invites readers to generate their own ideas of what stem cell research could lead to and how they could uniquely apply the information, leaving them with the idea of what their own journey in STEM might look like. Overall, this portion of the text reiterates the key points covered in the previous section on stem cell research and serves to demonstrate to the reader the need for continued support and discovery across all areas of science and research.

3.2.2 Content Design: Illustrations

Over the course of the project, the visuals were developed to further connect the text to the reader, with the goal of creatively demonstrating real and relevant science in a clear, concise, accurate, and manageable format (Figure3-2). With science, communication, and creativity colliding at the forefront of design, images were created around a specific set of principles aimed at conveying key points from each page of text. Visuals throughout the book include representations of various types of cells, biological processes, concept diagrams, anatomical diagrams, interactive pages, medical illustrations, and even lab generated images. Illustrations with high levels of scientific accuracy are a key tool for communicating science in the book, helping to eliminate many of the common misconceptions of scientific processes and research. Illustrations also intentionally

highlight opportunities in STEM-related disciplines and their contribution to stem cell research, to highlight diversity within various associated careers. Towards this goal, the illustrations featured in the book encourage readers to identify their own potential for unique contribution in a stem cell research-related field by depicting unique and diverse individuals involved in the interdisciplinary work.

Finally, attractive illustrations for readers lend a positive and inviting tone to the book helping the readers develop an optimistic outlook and increased interest in stem cell research. Specific color/shape patterns were designated to represent key pieces of information found and depicted throughout the book. These illustration consistencies provide pattern-picture associations, allowing readers to visually form connections with information presented in the text and build on their connections as new information is presented over the course of the book. Overall, illustrations included in the book were designed to aid student engagement, provide a visual method for content delivery, and promote positive interest in scientific research to encourage greater understanding, clarity, and application while interacting with the book.

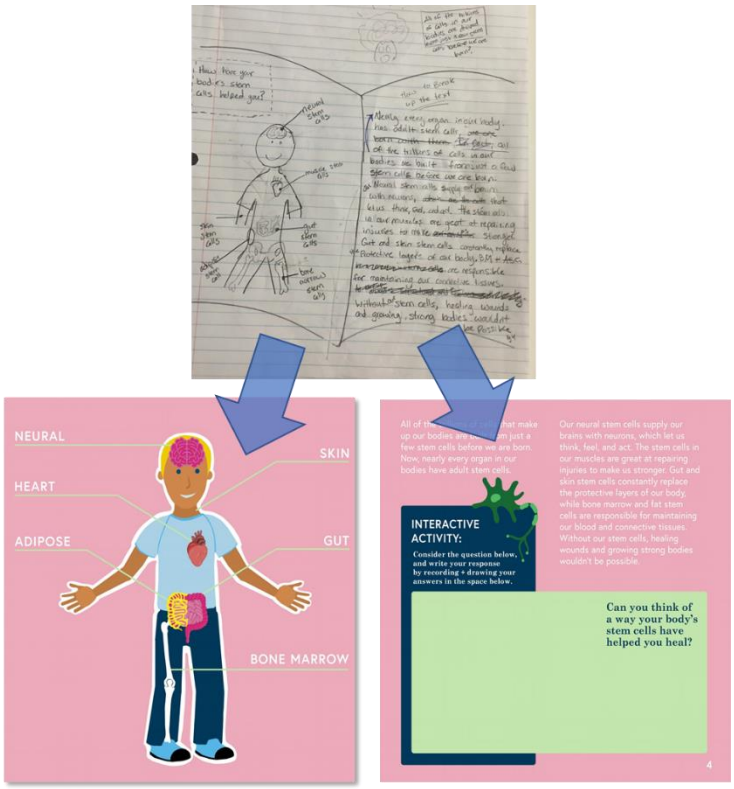


Figure 3-2: Illustration development in the book. Key points were originally determined in combination with text and outlined. Key points in the text were transformed into clear and concise images, in collaboration with the artist, for accurate and understandable visual communication.

3.2.3 Content Design: STEMQuests

One specific goal of the book was to provide an interactive tool that aided in identifying STEM topics and careers as interesting and relevant to each reader. Through specific engagement opportunities, displayed as STEMQuests, readers have the opportunity to think critically and creatively through guided activities (Figure3-3). STEMQuest activities allow students to recall, understand, apply, and analyze the content in an individualized manner that challenges students to understand how stem cells, research, and regenerative medicine can specifically impact their own lives.

STEMQuest activities in the book encourage students to focus on key information in the text and give the reader an opportunity to respond in their own personalized way. Each chunk, or informational section, of the book contains activities that aid students in identifying and sorting key information from each section in a specific way. This was intentionally designed and included as an effective and engaging tool for content delivery. Specifically, STEMQuest activities ask students to answer questions, draw, label, write, create informational lists, and identify key words. The inquiry-based learning format[49] encourages students to ask questions and provides a platform for students to explore specific topics discussed in the text. With the STEMQuests, students are active in learning the content and the activities can provide formative assessment material for educators that may incorporate the book into a formal educational setting.

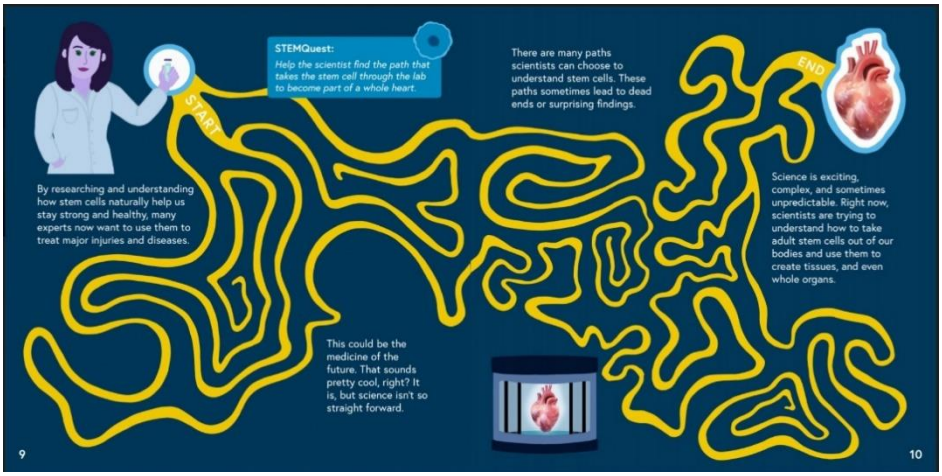


Figure 3-3: STEMQuests provide an interactive educational element. This figure demonstrates the incorporation of a maze into the scientific process, staring at understanding stem cells and then discovering the path to a stem cell-generated organ. Here, students can view stem cell research with a "start" and "finish" providing an overview to the importance of stem cell research. The maze demonstrates the many paths, dead ends, challenges, and questions associated with scientific research and relevant findings. Illustration credit: Maddie Dearman.

3.2.4 Glossary/Additional Resources

The glossary and additional resources included in the book are designed to serve as supporting material for book content and to aid readers in understanding important concepts. Definitions are provided in this section for bolded vocabulary words that are found throughout the book. Definitions can be read and applied to the pages of the book to provide better understanding of the concepts and key points that are depicted and/or described.

Additional resources are also included for advanced audiences that are interested in further exploring and learning about various topics of stem cell research, as well as resources for educators that can be incorporated in various educational settings. This section provides a more detailed review of stem cells, their sources, and current and potential roles in clinical applications. The printed format of the book is limited to the amount of additional resources able to be included, therefore, the additional resources in the book are also extended to a web page, easily accessible by readers. The webpage offers links to other websites for outside information and provides answers and tips for STEMQuests. The glossary and resources, and its extended addition, aid readers in further exploration and understanding of relevant stem cell topics, research, careers, applications, and involvement and aids the goal of the book to address common stem cell misconceptions and develop STEM interests in young audiences.

3.2.5 Modifications and Dissemination

Idea development began with a search for available stem cell resources targeting and early-middle aged education, including books, lesson plans, videos, websites, etc. With the identification of areas in education lacking stem cell content, it was decided to develop

materials that would address the lack of available content for early education of stem cell biology and research. Content development originated with an outline of essential information developed to focus the theme of the book and connect with the specific objectives. In preparation to collaborate with the artist to develop illustrations to represent the key themes, content was designated to pages for a full framework of the book. Text and Illustrations were adapted when initial versions of the book were not able to achieve original goals due to the target audience being too young. Content and design modifications were continual throughout the development process. Essential details were added to the text and figure content to strike an informative yet user friendly balance. Much time was spent communicating and working closely with the artist to ensure the scientific accuracy and clarity of the illustrations; figures, images, and STEMQuests were added to aid realization of book themes and objectives. The process of reviewing, critiquing, and editing the completed content was aided by professionals, both inside and outside of stem cell research, who analyzed the accuracy and effectiveness of the book. Full versions of the book were sent to various researchers, doctors, educators, and other colleagues to specifically analyze content and illustration accuracy and offer feedback and possible modifications to enhance the clarity, communication, flow, engagement, and overall effectiveness and appeal for our target audience.

The book was published in May of 2020 with dissemination intended to begin immediately. Thus far, the book was featured at the Masur Museum of Art for a VISTA event during the exhibition of Art and Nature in the Post-Digital World. The book was also displayed and discussed during a panel presentation at the 2020 Louisiana Tech Symposium for Visual Communication and Visual Literacy, where its design,

communication, artistry, and interactive characteristics were demonstrated and reviewed. Lastly, the book has also been featured in the 2020 Sigma Xi Annual Meeting and Research Conference STEM Art and Film Festival. The book was displayed as a collective piece of STEM 2D art to exhibit the combination of art, STEM research, and education, to enhance scientific communication.

Despite original plans to integrate the book into educational settings by visiting local schools, offering virtual and face-to-face readings, conferences, and on/off campus events where the book would be featured, complications due to shutdowns from the COVID-19 pandemic halted many of these plans. Over the 2020 summer, 35 books were dispersed to high school aged students participating in a virtual Louisiana Gear UP camp on visual communication. More recently 35 books were given to students in the Monroe, LA MLK Jr. Middle School after school program. Plans are in place to continue dissemination routes during the upcoming school year and beyond, where efforts will be made to send books to students and host virtual readings and discussions, among other potential opportunities.

3.3 Results

3.3.1 VISTA Webpage

Though the book was our main objective for communicating science and attracting young readers to stem cell research, its format contains a finite amount of information. To promote continued learning and provide further resources, a web page for the The Journey of a Stem Cell has been developed as a tool to both, expand the resources in the book and

serve as a collective place that offers continued, new, and unique educational resources to the public (Figure3-4).

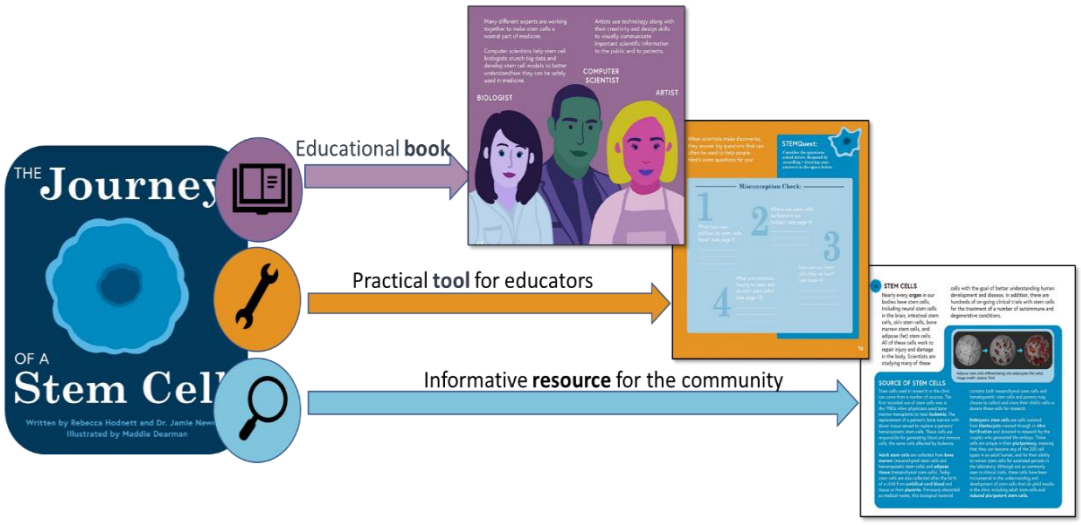


Figure 3-4: The Journey of a Stem Cell was developed as a multipurpose educational resource to share scientific information. This figure shows the versatility of the book, as it serves as an educational book for students, a practical, interactive tool that can be used by educators, as well as an informative resource for the community by providing content and links for furthered, and self-guided learning.

With the link to the VISTA webpage located in the Glossary of the book for additional resources, anyone interacting with The Journey of a Stem Cell will have access to STEMQuest answers, detailed explanations, and tips for students completing the activities. Links to outside resources that lead readers to scientific findings and information from the National Institute of Health, educational websites providing information for students and activities to help educators peak student interest, as well as information on current clinical trials involving stem cells. In addition, the VISTA website aids in highlighting unique careers in science communication, stem cell research, and regenerative medicine. Collectively, The Journey of a Stem Cell webpage disseminates information beyond the book, aids in attracting young readers to STEM based careers, and offers

educational links and STEMQuest explanations to support the original goals of the book and encourage project expansion.

CHAPTER 4

CONCLUSION

4.1 Understanding Mechanisms of Regulation

Elucidation of the influence of MED12 on the Notch signaling pathway provides distinct insight into the mechanisms governing the role of these factors in human adipose-derived stem cells. By investigating the relationship between MED12, Notch1, and Notch3 activity in the self-renewal of hASCs, this project was able to identify a novel regulatory influence by MED12 on the Notch signaling pathway. Specifically, we have observed that MED12 regulates the activation of the Notch3 signaling pathway during self-renewal. The influence of Notch signaling on hASCs, namely the Notch1 and Notch3 pathways, drives specific cell states *in vitro*. By providing evidence of novel relationships between MED12 and the Notch3 signaling pathway, we introduce the complex cross-talk involved in the transcriptional regulation and cell fate of hASCs.

We also concluded that due to our observed lack of expression of Notch1 during hASC self-renewal and the absence of influence on Notch1 when MED12 was reduced, there was no significant link between MED12 regulation and the Notch1 signaling pathway. This data provides support for previously published research identifying two distinct roles for the Notch1 and Notch3 pathway and suggesting individualized mechanisms of regulation for each. Though this project did not analyze other potential regulators for the levels of Notch1 expressed in hASC self-renewal, the data presented here

indicates the need for furthered investigation of the novel interactions and regulators of the Notch1 pathway and its own unique influence on cell state.

Elucidating unknown roles of major regulators, like MED12, in cell fate and gaining knowledge of pathway interactions and the less well characterized non-canonical Notch pathway that contributes to cell state is indispensable in understanding the biology of stem cells. This data, more explicitly, offers explanation into a specific mechanism that controls the maintenance and renewal of hASC stem populations. This contribution lends greater potential for the use of human adipose derived stem cells in novel therapeutics and clinical applications, through the understanding and control of cell fate.

4.2 Communicating Science and Research Topics

In combination with this research uncovering novel mechanisms of transcriptional control in human stem cells, we have also demonstrated the components and process involved in effective communication of this science to public audiences. The unique design of The Journey of a Stem Cell promotes a key requirement for the advancement of stem cell research and regenerative medicine: support for further investigations of the mechanisms driving stem cell characteristics. This is accomplished by supplying a tool for effectively communicating the relevance, impact, and elements of investigation involved in stem cell research to inform and encourage others to pursue research in the future. This tool, equipping audiences with fundamental knowledge of stem cells, aids in the addressal of common misconceptions regarding clinical applications and provides a unique account of information and resources derived by experts in the field, to the public, otherwise not found.

As a science communication tool, the dissemination of The Journey of a Stem Cell has engaged public audiences with real and relevant science topics that promote interest and understanding. Encouraging the audience to explore various STEM related careers through elements of individualism has attracted students to the possibilities of interdisciplinary professions in science and related fields. Because of this influence, The Journey of a Stem Cell encourages the next generation of scientists, doctors, researchers, artists, etc. Collectively, the culmination of this project has been a valuable and educational tool in science communication and an innovative model for the future of interdisciplinary works and the communicative advantages provided by the collaboration between art and science.

4.3 Future Work

The insight gained from the research presented here in combination with new inquiries exposed for continued investigation, collectively expresses the need for further elucidating the individual relationships and interactions of signaling and transcriptional control. Because this data suggests a novel regulatory role for MED12 on the Notch signaling pathway, it is necessary to investigate in greater detail exactly how MED12 controls the activation of the Notch3 pathway and what specific interactions are involved. This research offers a greater insight into the mechanisms of action that initiate and inhibit the Notch signaling pathways in hASCs.

Here we demonstrate that MED12 influences the activation of Notch3 signaling. This along with previous knowledge of MED12 being linked to the expression of Wnt/β-catenin responsive genes, including the Jagged1 ligand of the Notch3 pathway, suggests that it is necessary to consider potential influences of MED12 on the expression of the

ligands responsible for activating Notch. Subsequently, it is crucial to also acknowledge that a second mechanism responsible for the activation of the Notch signaling pathway, specifically the release of the Notch intracellular domains, is accomplished through protease cleavage of the intramembrane portion of the receptor. Therefore, future investigations may also consider the possibility that MED12 regulates the activation of the pathway via inhibition or interruption of the cleavage of the receptor. Elucidating any influence of MED12 on the expression or action of the γ-secretase and/or metalloprotease cleavage or the Notch specific ligands will provide insight to the control and activation of the Notch3 signaling pathway and associated consequences on cell state.

Future work using small interfering RNA in self-renewing hASCs, will demonstrate the effects of reduced MED12 expression on specific targets potentially co-regulating the activation of the Notch3 pathway. By reducing MED12 in self-renewing cells, changes in expression and activation levels of specific targets provides clues to the mechanisms used by MED12 to influence Notch3. By employing quantitative PCR methods and Western blot analysis, expression and protein levels for Notch ligands as well as the protease and secretase enzymes can be evaluated for significant changes in expression or activation, suggesting the direct influence of MED12. Further, co-immunoprecipitation techniques can be employed in combination with Western blot to discover direct protein-protein interactions resulting in the regulation of the Notch3 pathway by MED12.

Due to the focus of this project turning primarily to the Notch3 pathway, elucidating the mechanisms and effects from the lack of Notch1 expression demonstrated here is also a crucial next step in fully understanding specific interactions and regulatory mechanisms,

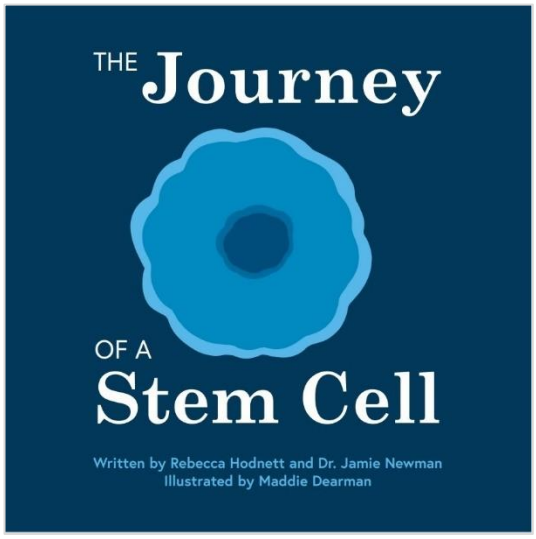
thus the effect of Notch1 on cell fate. Considering its varied expression level in self-renewal from Notch3, it would be beneficial to further validate the low of expression and activation of Notch1 in self-renewing cells as a first step to better understanding it's influence on cell state. Then, elucidating the regulators of Notch1 activation or inhibition and which specific interactions determine this will provide new information of the governing elements acting on the Notch1 pathway. Given its distinct role and known links to adipogenesis, investigating possible regulators affecting the activation and inhibition of this pathway will likely be most beneficial if performed in differentiating hASCs. Collectively, future investigations stemming from conclusions stated here provide a stride forward in understanding the transcriptional regulators, protein-protein interactions, and signaling pathways that govern cell fate. Discovering the unique systems and processes which determine the characteristics of stem cell state has and will continue to aid in determining the most effective therapeutic and clinical use of human adipose-derived mesenchymal stem cells supporting the future of regenerative medicine.

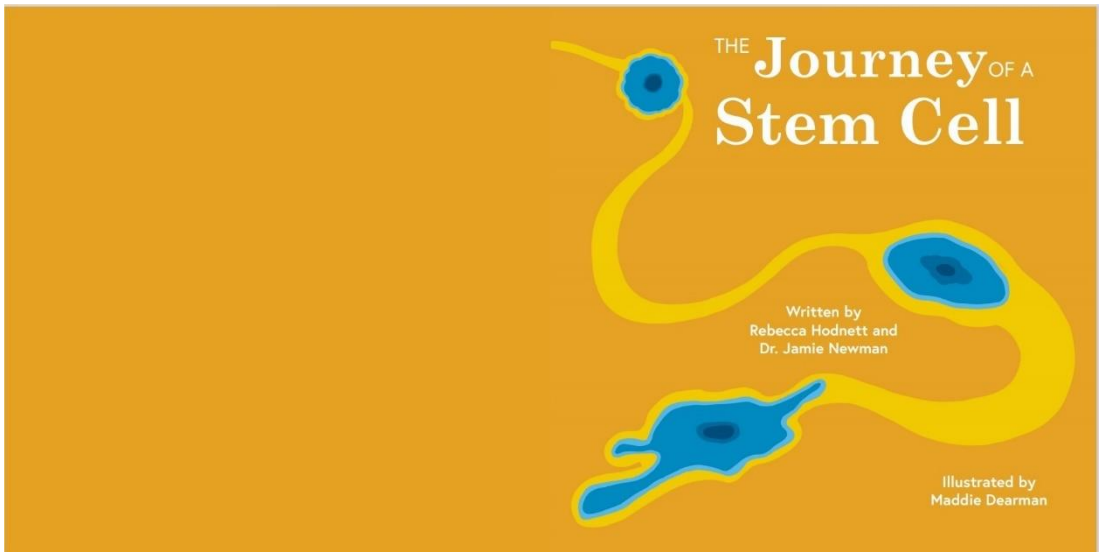
APPENDIX A

THE JOURNEY OF A STEM CELL

A.1 Pages of The Journey of a Stem Cell

A pdf formatted version of The Journey of a Stem Cell and its contents are presented in this section.





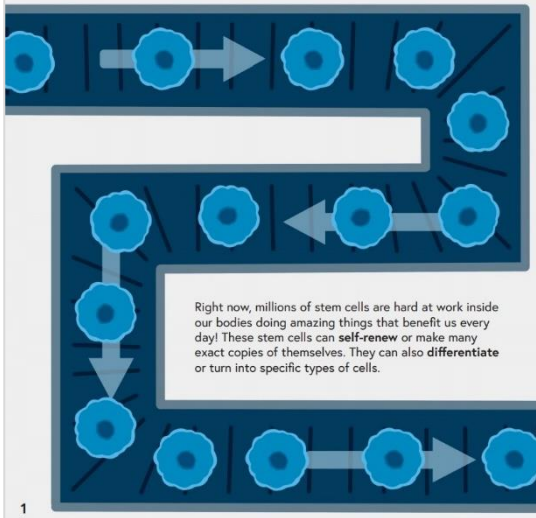
This book is dedicated to you – our reader who opened it to start on a journey of scientific exploration and education. The potential in new discoveries inspires all of us. With this book, we want the future of biology and medicine to inspire the next generation of scientists, doctors, engineers, artists, astronauts, and educators.

This book – created when people in different fields and with different interests worked together – was sparked by our passion for education and a desire that you'll find your own part in this emerging area of science and research.

Enjoy the journey!

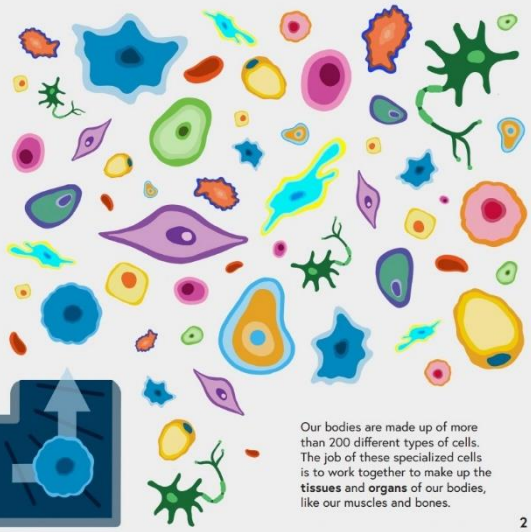
LOUISIANA TECH UNIVERSITY

ISBN: 978-1-7346259-1-2



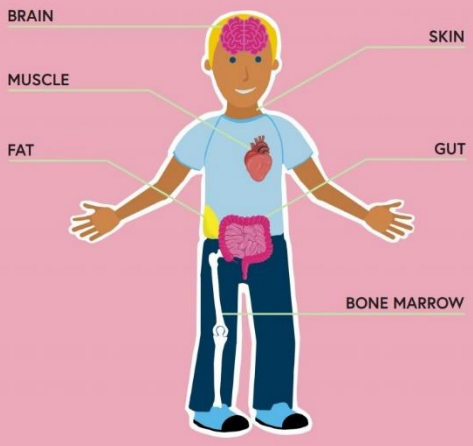
Right now, millions of stem cells are hard at work inside our bodies doing amazing things that benefit us every day! These stem cells can **self-renew** or make many exact copies of themselves. They can also **differentiate** or turn into specific types of cells.

1



Our bodies are made up of more than 200 different types of cells. The job of these specialized cells is to work together to make up the **tissues** and **organs** of our bodies, like our muscles and bones.

2



3

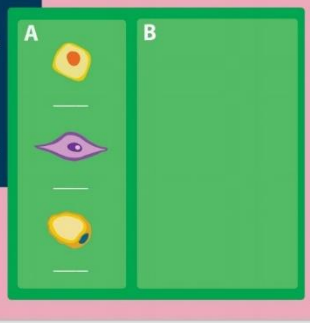
Before we are born, all of the trillions of cells that make up our bodies are built from just a few original stem cells. Now, nearly every organ in our bodies still have stem cells. We call these adult stem cells.

STEMQuest:

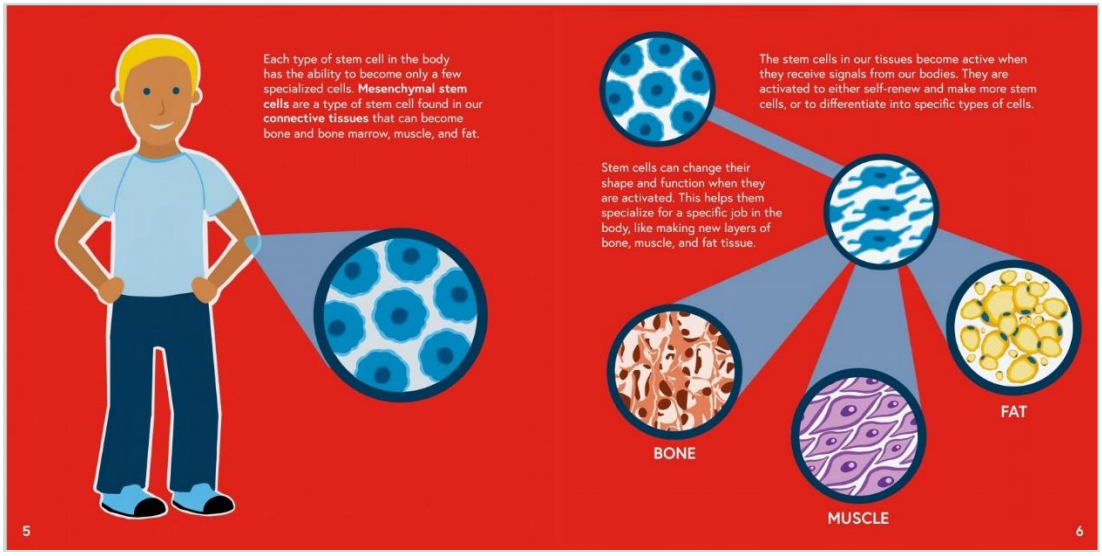
Quest A: Three types of the cells that are found in our body are pictured to the right: fat cells, muscle cells, and skin cells. How many of these three cell types can you find on page 2? Turn back to page 2 and record the number of cells in the space below each image.

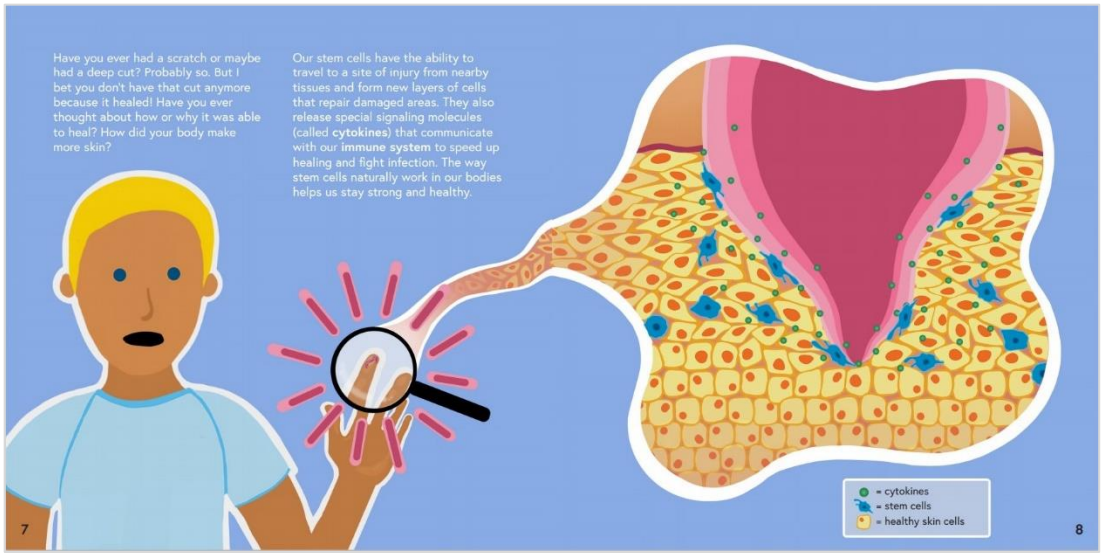
Quest B: Did you see any other cells that look familiar? What other types of cells can you identify on page 2? Take a minute to sketch and label those here.

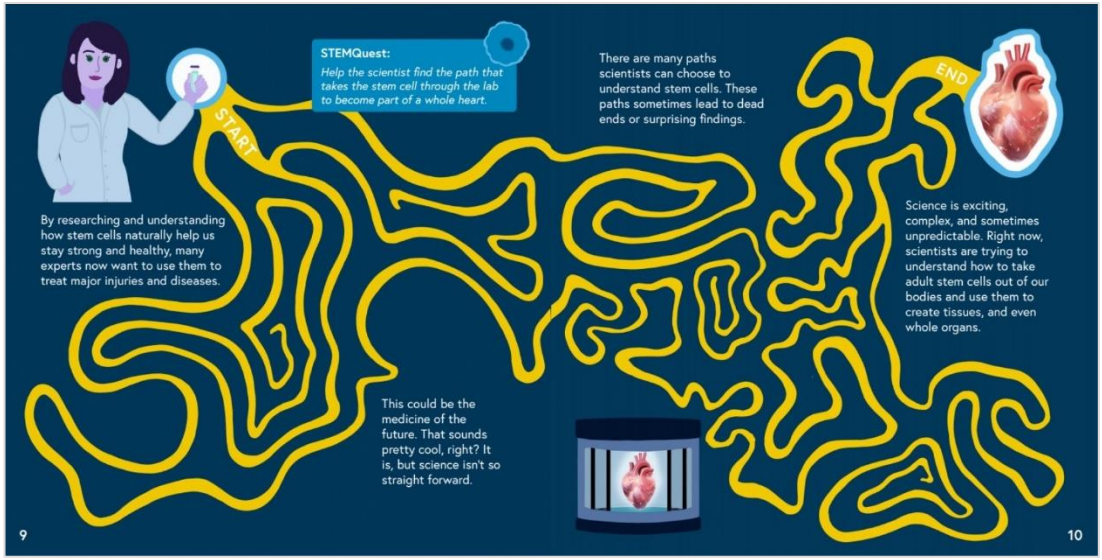
Stem cells in our brains make neurons, which let us think, feel, and act. The stem cells in our muscles are great at repairing injuries and make us stronger. Gut and skin stem cells constantly replace the protective layers of our bodies, while bone marrow and fat stem cells are responsible for maintaining our blood and connective tissues. Without our stem cells, healing wounds and growing strong bodies wouldn't be possible.



4


Each type of stem cell in the body has the ability to become only a few specialized cells. **Mesenchymal stem cells** are a type of stem cell found in our **connective tissues** that can become bone and bone marrow, muscle, and fat.
5
The stem cells in our tissues become active when they receive signals from our bodies. They are activated to either self-renew and make more stem cells, or to differentiate into specific types of cells.
Stem cells can change their shape and function when they are activated. This helps them specialize for a specific job in the body, like making new layers of bone, muscle, and fat tissue.
BONE
MUSCLE
FAT
6


Have you ever had a scratch or maybe had a deep cut? Probably so. But I bet you don't have that cut anymore because it healed! Have you ever thought about how or why it was able to heal? How did your body make more skin?
Our stem cells have the ability to travel to a site of injury from nearby tissues and form new layers of cells that repair damaged areas. They also release special signaling molecules (called **cytokines**) that communicate with our **immune system** to speed up healing and fight infection. The way stem cells naturally work in our bodies helps us stay strong and healthy.
7
= cytokines
= stem cells
= healthy skin cells
8


STEMQuest:
Help the scientist find the path that takes the stem cell through the lab to become part of a whole heart.
START
END
There are many paths scientists can choose to understand stem cells. These paths sometimes lead to dead ends or surprising findings.
By researching and understanding how stem cells naturally help us stay strong and healthy, many experts now want to use them to treat major injuries and diseases.
This could be the medicine of the future. That sounds pretty cool, right? It is, but science isn't so straight forward.
Science is exciting, complex, and sometimes unpredictable. Right now, scientists are trying to understand how to take adult stem cells out of our bodies and use them to create tissues, and even whole organs.
9
10

Different experts are working together to make stem cells a normal part of medicine. All of these experts use knowledge and creativity to solve problems in new ways. They often work together to learn procedures, gain new information, and develop ideas.

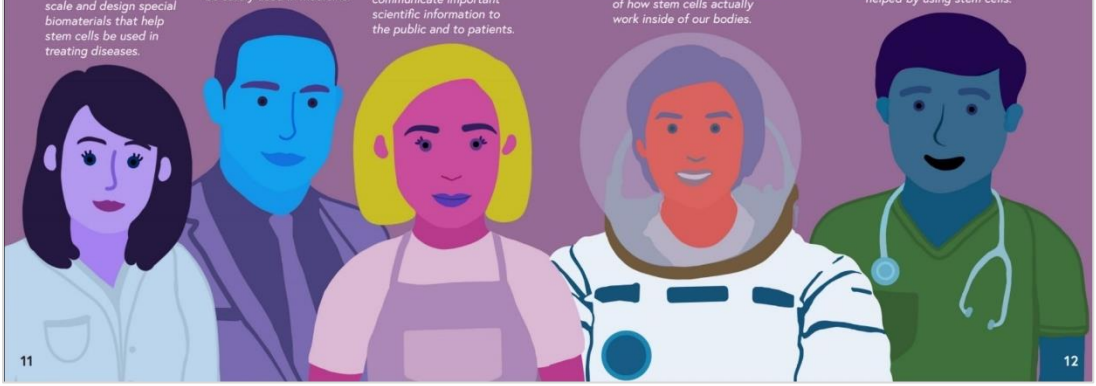
Biomedical engineers *can study how things work on a very small scale and design special biomaterials that help stem cells be used in treating diseases.*

Computer scientists *help stem cell biologists crunch big data and develop models to better understand how they can be safely used in medicine.*

Artists *use their creativity and design skills to communicate important scientific information to the public and to patients.*

Astronauts *working at NASA even perform stem cell research in outer space to provide a better understanding of how stem cells actually work inside of our bodies.*

Doctors *work directly with patients to apply treatments and understand how diseases and medical conditions are being helped by using stem cells.*



Scientists who study human adult stem cells in labs use cells from individuals who donate small amounts of their fat, bone marrow, and other tissues. These cells are **cultured**, or grown in controlled environments outside the body, for experiments that use special methods to explore important stem cell characteristics, like their ability to self-renew and differentiate into specific types of cells.

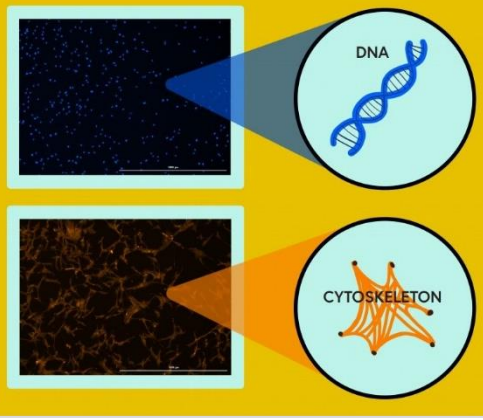
The information that comes from the research in these labs is used in the scientific and medical communities to provide clues on how stem cells work and how the cells can best be used in medicine.

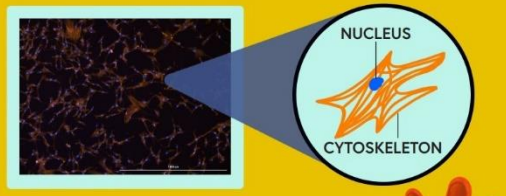
When scientists make discoveries, they answer big questions that can often be used to help people. Here are some big questions for you:

STEMQuest:
Consider the questions asked below. Respond by writing your answers in the space below.

1. What two main abilities do stem cells have? *(see page 1)*
2. Where can stem cells be found in our bodies? *(see page 4)*
3. How can stem cells help us heal? *(see page 7)*
4. What are scientists hoping to learn and do with stem cells? *(see page 10)*

Stem cells have many moving parts that control what they do in our body. Scientists have discovered that one main structure responsible for that control is DNA. Like a book of instructions, DNA is found in the headquarters of the cell, called the **nucleus**, and contains important information on how each cell needs to function. There is also a structure that provides shape to the cells, called the **cytoskeleton.** The shape of a cell is important because it helps to determine its job inside the body.





Stem cells can even send signals to communicate with each other. Signals can come from a neighboring cell or from a cell's **environment.** These signals may target the DNA inside the cell's nucleus. Once a signal is detected, DNA can provide important instructions to help the cell change, grow, and specialize as the body needs. These are just a few of the many important parts of stem cells that are being explored today.

STEMQuest:
The DNA inside each of our cells works like a book of instructions the cell can read. The messages in our DNA that help our cells function are hidden in over 6 billion letters, known as nucleotides, that make up the special code of instructions. Can you locate (circle) the following key words from the text hidden in these lines of DNA code:

Cytoskeleton	Code	Target
DNA	Instructions	Change
Signal	Nucleus	Environment

A C T G L C A T T C T A A T G A A G E N V I R O N M E N T A G
T G A C A T A I N S T R U C T I O N S G T A E A G A T G E C A
A C H A N G E G G T A C T A G T G C A G A C D T T G C G G C T
T G A T G C T C C A T G C Y T O S K E L E T O N A A C G R C A
G G T C I A C T T G G C D N A G T G A C A G C T A G C A A G C
C C A G S T G A A C C G C T T G N U C L E U S G A T C G T T C

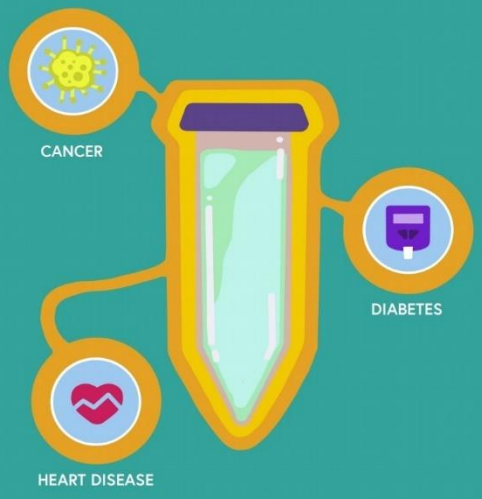
With the knowledge that DNA is the book of instructions that cells use, scientists are working to map out the ways stem cells communicate so they can understand how each important piece connects. For stem cell scientists, these are like little clues in a big puzzle.

How can stem cells change their shape? How do stem cells send messages to other stem cells? What do they say to each other and the cells around them? How does this affect the body? What does this mean for the future of science and medicine?

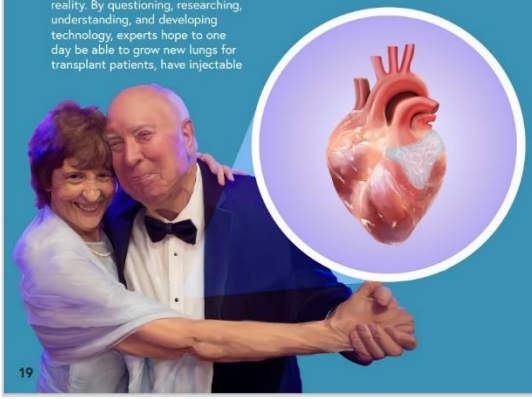
If scientists can map out how stem cells work both inside and outside of our bodies, stem cells could one day be used for great medical advances, even new treatments for major diseases like cancer, diabetes, and heart disease. There's so much to explore!

STEMQuest:

Take a second to think of people you know that have been affected by heart disease, cancer, diabetes or another major disease or injury. How do you think stem cells will be able to help them in the future?



The idea that scientists can use our own stem cells to grow new tissue and whole organs to replace damaged tissues and help cure disease is an area of science called regenerative medicine. The goal of many stem cell scientists working today is to make regenerative medicine a reality. By questioning, researching, understanding, and developing technology, experts hope to one day be able to grow new lungs for transplant patients, have injectable cures for joint diseases, and have functional tissue patches for people with heart problems. Can you imagine how this might change the world? The future of stem cells and regenerative medicine is bright, but for now, there is still much to do.



STEMQuest:

Choose one of the experts found on pages 11 and 12 or choose any other expert that may work in the field of regenerative medicine. Draw what you would look like if you were that expert and how you would be working in regenerative medicine.

Stem cells are some of the tiniest pieces of our bodies, but are designed to do big things, just like you!

So, what's next? What do you think the future of stem cells will look like? The future of medicine and science? How could you be a part of it? What will your journey look like?

GLOSSARY

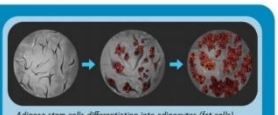
The Glossary portion is included to provide supporting material for the content of this book and offer sources that further explain and describe relevant stem cell information. Intended for more advanced audiences, this section aims to provide a more detailed review of stem cells, their sources, and their current and potential clinical applications to encourage understanding and involvement. Definitions to bolded scientific terms throughout the book are also included in this section.

STEM CELLS

Nearly every **organ** in our bodies have stem cells, including neural stem cells in the brain, intestinal stem cells, skin stem cells, bone marrow stem cells, and adipose (fat) stem cells. All of these cells work to repair injury and damage in the body. Scientists are studying many of these cells with the goal of better understanding human development and disease. In addition, there are hundreds of on-going clinical trials with stem cells for the treatment of a number of autoimmune and degenerative conditions.

SOURCE OF STEM CELLS

Stem cells used in research or the clinic can come from a number of sources. The first recorded use of stem cells was in the 1980s when physicians used bone marrow transplants to treat **leukemia**. The replacement of a patient's bone marrow with donor tissue served to replace a patients' hematopoietic stem cells. These cells are responsible for generating blood and immune cells, the same cells affected by leukemia.



Adipose stem cells differentiating into adipocytes (fat cells). Image credit: Jessica Trinh

Adult stem cells are collected from **bone marrow** (mesenchymal stem cells and hematopoietic stem cells) and **adipose tissue** (mesenchymal stem cells). Today stem cells are also collected after the birth of a child from **umbilical cord blood** and tissue or from **placenta**. Previously discarded as medical waste, this biological material contains both mesenchymal stem cells and hematopoietic stem cells and parents may choose to collect and store their child's cells or donate those cells for research.

Embryonic stem cells are cells isolated from **blastocysts** created through in **vitro fertilization** and donated to research by the couples who generated the embryo. These cells are unique in their **pluripotency**, meaning that they can become any of the 200 cell types in an adult human, and for their ability to remain stem cells for extended periods in the laboratory. Although not as commonly seen in clinical trials, these cells have been instrumental in the understanding and development of stem cells that do yield results in the clinic including adult stem cells and **induced pluripotent stem cells**.

STEM CELL RESEARCH

Cells are currently being used in research labs to understand development and human disease. Broadly, investigations include the **clonal expansion** and **self-renewal** of various stem cells, **cell state** regulation, and differentiation potential. Research on cell state allows scientists to understand the mechanisms the cells use to maintain stem cells populations via self-renewal, while understanding the ability and mechanisms stem cells use to differentiate and become specialized cells, will provide understanding for the uses and potential clinical applications of stem cells.

The overarching goal of stem cell research is the employment of stem cells in **regenerative medicine**. Regenerative medicine deals with engineering our bodies' own cells in a way that allows them to be harvested and to regenerate specific tissues, replace damaged organs, and/or restore normal bodily functions. To date, cells are also being used in clinical trials to treat injuries, autoimmune diseases, and degenerative health conditions such as: Chron's disease, Parkinson's disease, Alzheimer's, multiple sclerosis, rheumatoid arthritis, traumatic spinal cord injuries, various cancers, and etc.

**DAPI staining is a fluorescent staining technique used to microscopically view chromosomes (DNA) in live or fixed cells. In stem cell research, DAPI staining often aids in analyzing cellular and nuclear morphology in a cell culture: cellular characteristics like the shape, form, structure, and size of cells.

Phalloidin staining is similarly used to identify cellular morphology. Phalloidin staining, specifically, fluorescently labels the actin filaments in a cell, which largely aid in providing structural support for cells and help determine their shape.

Analyzing both nuclear and cellular morphology in a cell population provides key information about cellular characteristics that help determine stem cell state and function.

GLOSSARY

Adipose-derived stem cells (hASCs): Adult stem cells sourced from adipose or fat tissue. hASCs are easily retrieved with small volumes of fat and offer significant self-renewal and multilineage differentiation making them optimal for use in research and potential clinical applications.

Adult stem cells: Also referred to as somatic stem cells, these are cells that are found in many organs and differentiated tissues that have a limited capacity for self-renewal and differentiation (multipotent).

Biomaterials: Biomaterials may be natural or synthetic and are used in medical applications to support, enhance, or replace damaged tissue or a biological function. Metals, ceramics, plastic, glass, and even living cells and tissue all can be used in creating a biomaterial. They can be reengineered into molded or machined parts, coatings, fibers, films, foams, and fabrics for use in biomedical products and devices. These may include heart valves, hip joint replacements, dental implants, or contact lenses. They often are biodegradable, and some are bio-absorbable, meaning they are eliminated gradually from the body after fulfilling a function.

Bone marrow-derived stem cells: A multipotent population of cells found in the bone marrow that are different from blood cells and include hematopoietic and mesenchymal stem cell

Cell Culture: The growth and maintenance of cells outside the body for the purpose of research or medical treatments. Cells are maintained in a medium that provides an artificial environment for cell proliferation and/or differentiation.

Cell: The structural and functional unit of living organisms; often known as the basic unit of life. Cells contain tiny structures called organelles which allow them to provide structure, nutrient uptake, growth, and other important functions necessary for life. Cells group together to form tissues, which in turn group together to form organs, then living organisms.

Cell: The structural and functional unit of living organisms; often known as the basic unit of life. Cells contain tiny structures called organelles that provide structure, nutrient uptake, growth, and other important functions necessary for life. Cells group together to form tissues, which in turn group together to form organs, then living organisms.

Cell state: Cell state refers to the current physiological condition of a given cell, often observed through cell behavior; when a cell transitions from one type to another. Cell state includes proliferation states, metabolic profiles, differentiation, and is often regulated, or influenced, through gene expression, i.e., an adipose stem cell differentiating or transitioning into an adipocyte, or fat cell.

Clonal expansion: Refers to a single cell dividing and producing identical daughter cells; occurs so that a stem cell population can be maintained or increased. *See also* **self-renewal.**

Connective tissue: Connective tissue binds to, provides cohesion for, and anchors other tissues in the body to promote structure and internal support. It largely consists of ground substance, fibers, and cells to make up much of the extracellular matrix. Examples include adipose, cartilage, bone, tendons, and blood.

Cytokines: A broad family of signaling molecules that help control cell division and differentiation within the immune system, often associated with functions that include mediating immunity, cell communication, inflammation, and repair. Cytokines can be released by immune cells or cells surrounding a site of injury to promote new cell growth, differentiation, and healing.

Differentiate: The ability of stem cells to specialize for specific jobs in the body. Stem cell differentiation includes changes in gene expression, cell shape, structure, size and cellular function. *Also called* **differentiation.**

DNA: Deoxyribonucleic acid is found in the nucleus of cells. DNA is the genetic code of a cell, containing genes that provide instructions to make the RNA and proteins responsible for cellular function and, in turn, is the blueprint for the cellular structure, materials, and activities needed for the functioning of our bodies.

Embryonic stem cells (ESCs): Embryonic stem cells are isolated from the inner cell mass of a 5-day pre-implantation embryo and are capable of dividing without differentiating for a prolonged period in culture. ESCs are also known to be pluripotent, meaning that they can become any cell or tissue of the ectoderm, endoderm, or mesoderm germ layer. Human ESCs are only isolated from embryos that were generated through in vitro fertilization (IVF) and donated by the parents through a process of informed consent.

Environment (cellular): Known as the stem cell niche, it is the specific location within where populations of stem cells are located. This specified microenvironment provides signals that help govern stem cell decisions; it aids in the maintenance of local stem cell populations and determines stem cell fate.

Hematopoietic stem cells (HSCs): A stem cell that gives rise to all red and white blood cells and platelets. These cells are found in bone marrow and umbilical cord blood.

Induced pluripotent stem cells (iPSCs): Stem cells generated from mature adult (somatic) cells. In iPSCs, the specialized functions of adult cells is reversed by manipulating expression levels of specific regulatory controls in the cell; stem cell characteristics are restored.

In vitro fertilization (IVF): A widely used medical procedure where an egg is fertilized by sperm outside of the body; often part of assistive reproductive treatments intended to achieve pregnancy.

Immune System: The body's natural defense system against invading pathogens. It is divided into the innate immunity and acquired immunity subsystems, consisting of cellular components, mostly white blood cells, that circulate around the body via blood and lymphatic vessels. The immune system also plays a role in wound and injury healing.

Leukemia: A form of cancer that effects a patient's blood cells; originating from their blood-forming tissues like the bone marrow and lymphatics. Leukemia affects a patient's red and white blood cells, platelet forming cells, and often requires a treatment regimen that involves a stem cell transplant to rebuild healthy bone marrow.

Mesenchymal stem cells (MSCs): These are non-blood adult stem cells from a variety of tissues including bone marrow, adipose tissue, and umbilical cord blood.

Nucleus: A membrane-bound organelle that contains a cell's genetic material, or DNA. Its primary function is to house the DNA, selectively regulating the molecules that move in and out, to regulate genetic activity and control cellular function.

Organ: A group of tissues collectively performing a specific function within a living organism. Examples include heart, kidney, and lungs

Pluripotency: "Potency" refers to the ability of a stem cell to differentiate into various cell types. Cells with greater potency can differentiate into a greater number of cell types. "Pluripotency" is the specific ability for a stem cell to become any of the 200 cell types within the body, i. e., pluripotency is exhibited by embryonic stem cells.

Regenerative Medicine: Regenerative medicine is a broad field that includes tissue engineering but also incorporates research on self-healing – where the body uses its own systems, sometimes with help from foreign biological material to recreate cells and rebuild tissues and organs. The terms "tissue engineering" and "regenerative medicine" have become largely interchangeable, as the field hopes to focus on cures instead of treatments for complex, often chronic, diseases.

Self-renewal: The process of proliferating in an undifferentiated state, giving rise to more cells with the same characteristic stemness. This is regarded as one of the defining traits of stem cells, allowing the maintenance of stem cell populations indefinitely.

Stem cells: Cells with the ability to divide for indefinite periods of time in culture (self-renewal) and give rise to specialized cells (differentiation). There are several types of stem cells defined by their characteristics and tissue source.

Tissue Engineering: Tissue engineering evolved from the field of biomaterials development and refers to the practice of combining scaffolds, cells, and biologically active molecules into functional tissues. The goal of tissue engineering is to assemble functional constructs that restore, maintain, or improve damaged tissues or whole organs.

Tissues: A group of cells with similar structure and function that together make up organs. Examples include muscle, nervous, and connective tissues.

Umbilical cord stem cells: Stem cells collected from the umbilical cord at birth that can produce all of the blood cells in the body.

For more information, a list of resources, and answers to the activities in the book visit www.latech.edu/vista

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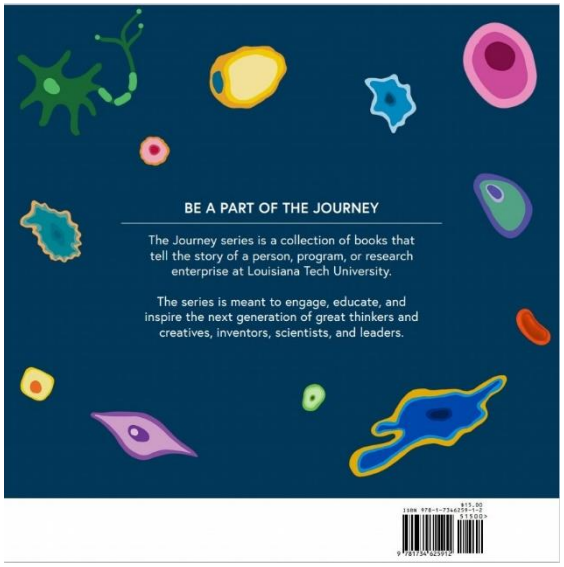
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The Journey series is a collection of books that tell the story of a person, program, or research enterprise at Louisiana Tech University.
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