

Regenerating Muscle: Characterization and Transplantation Potential of a Novel Cell Population

Field of Interest

In a society where a significant portion of health issues stem from degenerative disease, an understanding of the underlying mechanisms at the most fundamental molecular and cellular levels is vital in developing effective treatments. The branch of science seeking to achieve this understanding is known as regenerative biology, which aims to restore structure and function to damaged tissue by understanding and recapitulating the events occurring during embryonic development, when these structures are first formed.

Specifically, I am interested in the formation of skeletal muscle (myogenesis), and for my University Scholars Project, I hope to characterize a novel cell population of myogenic progenitor cells (muscle precursors) which are deficient in both MyoD and Myf5 genes, to determine their potential use for therapeutic purposes. Insight into their regenerative capacity through analysis of differentiation potential and fusion abilities may serve as a basis for treatment of neuromuscular diseases such as Duchenne Muscular Dystrophy.

I first garnered an interest in regenerative biology after taking MCB 4219 (Developmental Biology) my freshman year. Fortunate to be able to take advanced upper level classes early on in my undergraduate career, I took advantage of the opportunity: I loved the challenging environment where I could exercise my critical thinking skills. However, I soon came to realize that the classroom and textbook had its limits, and I yearned for more. The following summer, I obtained a position as an undergraduate researcher in a Developmental Biology lab at Yale University and discovered my love for research. The experience was intellectually stimulating, and I was able to apply what I had learned in the classroom to the lab, and learn a lot more in the process.

My interest in muscle biology stems from my experiences as an athlete as well as my interest in sports medicine, and is further supplemented by my aunt's recent diagnosis with muscular dystrophy. Thus, continuing my involvement with research through Dr. David Goldhamer's lab this past summer (whose research is geared towards the genetics and stem cell biology associated with muscle development in mouse) was a perfect combination of my intrigue in muscles with my passions for medicine and research.

I have already completed most of the coursework for my double major in Biological Sciences and Molecular and Cell Biology. I hope to use my experience as a University Scholar to further enhance my critical thinking skills and pursue my passion for research and regenerative biology before going on to medical school. With an increased push towards translational medicine in today's society, it is important for future physicians to have an understanding of basic science research, as during the prime of our careers, this technology will hopefully be at the forefront of clinical care.

Upon completion of my tenure as a University Scholar, I intend to produce a written thesis and poster presentation at Frontiers, with the ultimate goal of a journal submission.

Review of Literature

Myogenic Determination Factor (MyoD) is a member of the basic helix-loop-helix domain-containing myogenic regulatory factors (MRFs), whose expression is an ultimate result of complex transcriptional regulatory networks that control cell-autonomous activation of myogenesis in different regions of the embryo (Bryson et al, 2008). MyoD, along with another MRF, Myogenic Factor 5 (Myf5), plays an important role in the determination of skeletal myoblasts, and both genes function early on in the myogenic pathway and are largely functionally redundant (Rudnicki et al, 1993, Kablar et al, 1997). Either MyoD or Myf5 is

required for skeletal muscle formation, and inactivation of either gene alone results in relatively normal myogenesis after transient defects (Rudnicki et al, 1993). Conversely, combined inactivation of *Myf5* and *MyoD* results in a complete lack of skeletal-muscle formation (Rudnicki et al, 1993).

Myoblasts are committed, proliferating progenitors of the myogenic lineage that have the ability to differentiate into myocytes, which fuse to form mature multinucleated myofibers, subunits of skeletal muscle (Buckingham et al, 2003). This phenomenon is the basis for myoblast transfer therapy, involving transplantation of exogenous myoblasts which can contribute to the formation of new muscle fibers during repair and regeneration, and therefore result in genetic modification of the host muscle (Beauchamp et al, 1999). Although there are many benefits of using skeletal myoblasts in stem-cell based therapy, including ease of isolation through biopsy and *ex vivo* manipulation (Asakura et al, 2007), severe limitations currently exist including immune rejection of allogeneic donor cells, poor cellular survival after implantation, terminal differentiation in surviving cells, and limited proliferation and migration (Cao et al, 2005). In addition, growth in culture reduces the ability of myoblasts to engraft and survive due to the loss of an important subpopulation of cells that is capable of self-renewal (Kuang et al, 2007). Because of the challenges associated with transplanting myoblasts, it makes sense to look at cells with more primitive (stem-cell like) characteristics and determine their potential as a therapeutic cell type.

A recent paper published by Asakura et al (2007) showed that there was an increased survival of muscle stem cells lacking the MyoD gene after transplantation into regenerating skeletal muscle. Unlike myogenic progenitors, these stem cells, or satellite cells, are normally quiescent and reside between the myofiber basal lamina and plasma membrane, becoming

activated upon muscle injury to promote regeneration. Remarkably, MyoD null cells engrafted with a higher efficiency than wild type cells, showed self renewal properties, upregulated anti-apoptotic genes and stem cell markers, and down regulated apoptotic and muscle-specific genes. In the absence of MyoD, cells presented in a more primitive stem cell state, an intermediate between quiescent satellite and myogenic precursor cells (Asakura et al, 2007).

Because of the findings of Asakura et al (2007) in satellite cells, and the accepted functional redundancy of MyoD and Myf5, my hypothesis is that MyoD^{-/-}:Myf5^{-/-} double knockout progenitors will display more primitive stem cell characteristics than MyoD^{-/-} progenitors, and thus serve as a better transplantation base for therapeutic purposes because of an even more enhanced survival and fusion.

For clinical purposes, although these double null myogenic progenitors are not available for humans, isolated precursors in which the MyoD and Myf5 expression is chemically or post-transcriptionally suppressed could be generated to mimic MyoD^{-/-}:Myf5^{-/-} cell effects in transplantation, thereby providing a selective advantage for the expansion of stem cells (Asakura 2007). One possible approach is suggested in a recent paper by Kimura et al (2008) where MyoD expression can be initiated by the presence of exogenous Tamoxifen administration due to a transduced MyoD-ER(T) vector.

Methods

My project will include working with mice, and IACUC training and approval was obtained during July 2008 through addition to Dr. David Goldhamer's protocol. Through my experiences over Summer 2008 (30 hours/week) and this semester (20 hours/week), I have gained the necessary foundation in molecular techniques needed to perform the following experiments.

The first aim of this research project will involve breeding mutant mice to develop the double knockout phenotype. A breeding schematic utilizing heterozygotes for MyoD and Myf5, and a Cre-dependent fluorescent reporter mouse (obtained from Jackson Laboratories) will be followed to achieve the desired genotype in the fourth generation (already started). PCR analysis will be used to confirm the genotypes and the mutant embryos will be isolated by caesarian section thirteen days post coitum.

Cells competent to express MyoD will have recombined with Cre, and thus Cre-dependent reporter expression will result in YFP-labeled MyoD^{-/-}Myf5^{-/-} cells. These cells will be isolated enzymatically (using 0.5% Trypsin) from the limb buds of embryos, and then be purified through fluorescence-activated cell sorting (FACS) using standard protocol. The control mice, MyoD^{-/-} will also be bred and precursors will be isolated under the same conditions.

The second aim of this project will be to characterize the growth and differentiation potential of this novel cell population, as well as fusion potential with existing myoblasts. Cells will be cultured in various differentiation media, and will also be co-cultured with a population of C2C12 (wildtype) myoblasts in neutral medium. Fluorescence visualization of these cells through microscopy will be used to analyze fusion and integration. Myogenic potential will be classified by the expression of the cell marker Myosin Heavy Chain, and well as the ability to form multinucleated myofibers.

Characterization of this double knockout cell population is an important contribution to the understanding of muscle biology, and may help in the development of therapeutic agents for muscular disease. If time permits, I will also proceed with implantation of these precursors into injured adult mouse hindlimb, to determine *in vivo* effects of the ability of the cells to contribute to skeletal muscle repair.

Plan of Study

During Spring 2009, I plan to study abroad at the National University of Singapore (NUS) in their University Scholars Programme. As one of the top thirty universities in the world, in a city rich with culture and diversity, NUS will allow me to further my academic and personal development. Since freshman year, I have been planning my undergraduate curriculum and potential involvement in the University Scholars Program to include a semester abroad. At NUS, I will be able to take PNB 2250, a course relating to my project and offered at UConn only in the Fall, thus freeing up my Fall schedule for a graduate class. I will also take MCB 2610 and a history class to complete my General Education Requirements. In addition, I will pursue an Independent Study which will allow me to continue work on my University Scholars Project.

I will be spending Summer 2009 at UConn, working full time on my University Scholars Project in the lab of Dr. David Goldhamer, and completing medical school applications.

During Fall 2009, I plan to take MCB 5217, as it directly relates to my research with genetic manipulation in mouse cells, as well as another graduate class, MCB 5898. I will also take PNB 3263W towards my Neuroscience minor, and MCB 3989 to extend my knowledge of translational research. If the amount of work in three lab courses and two graduate courses is manageable, I will also take a business course, HSMG 3240. In addition, if my project is not moving along as expected, I may take MCB 4989 for more credits and spend more time in lab.

For my final semester, I plan to leave enough time to complete my project and write my thesis. I plan to take two graduate MCB courses with strong applications to my research, MCB 5426 and MCB 5280 (or 5243 if offered). In addition, I will take PSYC 2201 to complete my Neuroscience minor, and PNB 3262 to complete my Biological Sciences major. Having been a University Scholar, I will have completed a rigorous course of study and earned my bachelor's degree in Biological Sciences and Molecular and Cell Biology with a minor in Neuroscience.

Plan of Study Comparison Form

Spring 2009

Standard Plan of Study	Proposed University Scholar Plan of Study
PNB 2250 Animal Physiology (3)	PNB 2250 Animal Physiology (3)
HIST 1160 Asia and the Modern World (3)	MCB 2610 Microbiology (4)
NUSC 1170 Fundamentals of Nutrition (3)	HIST 1160 Asia and the Modern World (3)
MCB 2610 Microbiology (4)	MCB 3899 Independent Study (3)
	Possible: MCB 5683 Biotech. Seminar (1)

Fall 2009

Standard Plan of Study	Proposed University Scholar Plan of Study
MCB 3899 Independent Study (3)	MCB 5217 Biosynthesis of Nucleic Acids (3)
HSMG 3420 Intro. Healthcare Management (3)	MCB 4989 Honors Research (3+)
MCB 4989 Honors Research (3)	PNB 3263W Investigations in Neurobio. (3)
PNB 3263W Investigations in Neurobio. (3)	MCB 3989 Introduction to Research (2)
Elective	MCB 5898 Graduate Seminar Series (1)
	HSMG 3420 Intro. Healthcare Management (3)

Spring 2010

Standard Plan of Study	Proposed University Scholar Plan of Study
MCB 4997W Honors Thesis in MCB (3)	MCB 4997W Honors Thesis in MCB (3)
PSYC 2201 Drugs and Behavior (3)	MCB 5280 Advanced Cell Biology OR MCB 5432 Molec. Approaches to Developing Sys (3)
PNB 3263 Mammalian Endocrinology (2)	PSYC 2201 Drugs and Behavior (3)
Elective	PNB 3263 Mammalian Endocrinology (2)
Elective	MCB 5426 Gen. Engineering and Funct. Genomics (3)

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