Christopher Penton  
Ph.D. Candidate  

“**In Vitro Differentiation of Muscle Side Population Cells from Dystrophic Muscle Reveals Absence of Myogenesis and Implications for Hedgehog Signaling**”  

October 11th  
BRT 105  
1:00 PM
VITA

February 27, 1986 ................. Born in Kansas City, MO

June 2004 ....................... Conneaut High School

May 2008 ....................... B. A. Hiram College

2008 to present ................. Graduate Research Associate, The Ohio State University

AWARDS AND HONORS

Sigma Xi Grants in Aid of Research Awardee - 2013

Best Poster Award – The Research Institute at Nationwide Children’s Research Celebration - 2012

Best Poster Award – The Research Institute at Nationwide Children’s Research Celebration - 2011

COMMITTEE MEMBERS

Federica Montanaro, Ph.D.

Denis C. Guttridge, Ph.D.

Kevin Flanigan, Ph.D.

Santiago Partida-Sanchez, Ph.D.

FUTURE PLANS

Following graduation, I will start a joint postdoctoral position at Sanofi and The University of Arizona funded by a Muscular Dystrophy Association Bridge to Industry Postdoctoral Fellowship. In this position I will work in both academia and industry to develop novel therapeutics for Duchenne Muscular Dystrophy.
ABSTRACT

Duchenne muscular dystrophy (DMD) is a fatal neuromuscular disorder affecting 1 in 3500 live male births per year. The disease is caused by mutations in the dystrophin gene resulting in a lack of functional dystrophin protein at the membrane of both skeletal and cardiac muscles. Lack of dystrophin results in muscle fiber destabilization and breakdown, leaving the muscle in a chronic state of degeneration and regeneration. As the disease progresses however, regeneration ultimately fails resulting in the progressive replacement of muscle with fibrosis that correlates with patient loss of mobility in the second decade of life. Cell therapy is one of multiple therapeutic strategies being investigated for treatment in DMD to counteract these effects by increasing the regenerative capacity of the muscle and slowing the progression of fibrosis. While satellite cells and myoblasts have been used extensively in human trials, their efficacy has been limited. To overcome this, recent studies have turned to alternative cell populations residing in the muscle that have superior migratory and cell survival characteristics upon injection into limb muscles of the DMD mouse model, mdx. The muscle side population cells are a population of these alternative cells that have been shown to successfully engraft and regenerate dystrophic muscle in animal models. However, our understanding of muscle side population biology is limited due to a lack of specific in vivo markers and in vitro culture systems.

Here we show a novel in vitro system capable of supporting side population cell growth and differentiation. Using this system, we show that muscle side population cells lacking expression of extracellular markers CD31 and CD45 but expressing the fibro-adipogenic progenitor marker, Pdgfra, are highly myogenic giving rise first to pax7 expressing satellite cells and later into differentiated α-actinin expressing myotubes. We utilized this novel in vitro culture system to determine the myogenic capacity of SP cells isolated from either acutely injured or dystrophic SP cells. In contrast to wild type cells, side population cells isolated from dystrophic or acutely injured muscle display no myogenesis while differentiating into fibroblasts, adipocytes, and fibro-
adipogenic progenitor cells. These findings indicate that muscle damage and the state of the muscle environment direct the lineage commitment of the side population cells. Additionally, our results suggest that the cell fate switch of the muscle side population cells (SP) may play a role in dystrophic pathology. For example, cell fate switching of the side population may contribute to a reduced number of myogenic cells available for regeneration and in turn increasing numbers of fibro-adipogenic progenitors and fibroblasts responsible for fibrosis formation.

In order to better understand the mechanism responsible for the loss of myogenesis in mdx<sup>5cv</sup> SP cells, we analyzed gene expression of multiple signaling pathways functioning in cell fate specification. Our studies revealed that the hedgehog pathway (Hh) was significantly down regulated in SP cells isolated from mdx mice. Using the chemical Hh agonist, purmorphamine, we show that stimulation of Hh signaling successfully restores the myogenesis of mdx<sup>5cv</sup> SP cells.

Our findings in the SP cells suggested that Hh signaling may not be induced, as is the case during normal muscle regeneration. Analysis of dystrophic muscle revealed global down-regulation of the Hh pathway in the diaphragm muscle of dystrophic mdx<sup>5cv</sup> mice. Due to multiple positive effects of Hh signaling during myogenesis these findings suggest the lack of Hh induction may play a role in mdx<sup>5cv</sup> pathology. In order to test whether restoration of Hh signaling results in positive effects on mdx<sup>5cv</sup> pathology we treated mdx<sup>5cv</sup> mice with purmorphamine and analyzed multiple histological parameters. Treatment resulted in improvements in multiple disease parameters including increased resistance to fatigue, reduced immune cell infiltrate in limb muscles, and reduced central nuclei and Collagen 1 deposition in the mdx<sup>5cv</sup> diaphragm.

Collectively, the results in this dissertation show that SP cells isolated from either an actively regenerating or dystrophic muscle lack myogenic capacity. These results have implications for the use of autologous SP cell transplantation as dystrophic SP cells may not provide an optimal source of myogenic cells. Furthermore, we identify that Hh signaling serves to promote SP cell myogenesis and represents a potential mechanism and future therapeutic strategy in mdx<sup>5cv</sup> pathology.

RECENT ABSTRACTS AND PRESENTATION


