

Spring 5-13-2011

The Proliferation Of Vascular Smooth Muscle Cells Depends On Thioredoxin 1 Protein In A Model Of Pulmonary Hypertension

Viktoria Nelin

Western Kentucky University, viktoria.nelin421@topper.wku.edu

Follow this and additional works at: http://digitalcommons.wku.edu/stu_hon_theses



Part of the [Biology Commons](#), [Chemistry Commons](#), and the [Medicine and Health Sciences Commons](#)

Recommended Citation

Nelin, Viktoria, "The Proliferation Of Vascular Smooth Muscle Cells Depends On Thioredoxin 1 Protein In A Model Of Pulmonary Hypertension" (2011). *Honors College Capstone Experience/Thesis Projects*. Paper 329.
http://digitalcommons.wku.edu/stu_hon_theses/329

This Thesis is brought to you for free and open access by TopSCHOLAR®. It has been accepted for inclusion in Honors College Capstone Experience/Thesis Projects by an authorized administrator of TopSCHOLAR®. For more information, please contact topscholar@wku.edu.

THE PROLIFERATION OF VASCULAR SMOOTH MUSCLE CELLS DEPENDS ON
THIOREDOXIN 1 PROTEIN IN A MODEL OF PULMONARY HYPERTENSION

A Capstone Experience/Thesis Project
Presented in Partial Fulfillment of the Requirements for
the Degree Bachelor of Sciences with
Honors Graduate Distinction at Western Kentucky University

By

Viktoria E. Nelin

Western Kentucky University
2011

CE/T Committee:

Professor Nancy A. Rice, PhD, Advisor

Professor Kenneth Crawford, PhD

Professor Clay Motley, PhD

Approved by

Advisor

Department of Biology

Copyright by

Viktorija E. Nelin

2011

ABSTRACT

Pulmonary hypertension (PH) is a disease which manifests itself in the lungs of both adults and children. Vascular proliferation and remodeling are the hallmarks of PH and are found mainly in the pulmonary arterial smooth muscle cells (PASMC). The cause of PASMC proliferation and vascular remodeling in PH is poorly understood. Hypoxia, or low oxygen content, underlies many forms of PH. Hypoxia results in alterations in the redox balance of the PASMC. Therefore, the aim of this study was to examine the role of the thioredoxin system, an antioxidant system in the cell, in hypoxia-induced proliferation in PASMC. Protein was isolated from human PASMC which were exposed to either hypoxia (1% O₂, 5% CO₂, balance N₂) or normoxia (21% O₂, 5% CO₂ and balance N₂) to determine the protein levels of thioredoxin 1 and 2 (Trx1 and Trx2), thioredoxin reductase (TrxR), and thioredoxin interacting protein (Txnip), by Western blotting. Proliferation studies were also done by seeding 6 well plates with 10,000 PASMC per well, incubating in either normoxia or hypoxia for 5 days and counting viable cells using trypan blue exclusion. We found that in hypoxia the Trx1 protein levels were significantly greater after 48 and 72 hours of exposure than in PASMC grown in normoxia. We also found that PASMC proliferate more in hypoxia than in normoxia. To determine if Trx1 had a role in hypoxia-induced PASMC proliferation we knocked down Trx1 protein in the PASMC using specific siRNA, and treatment with the Trx1 siRNA completely prevented hypoxia-induced proliferation in PASMC. These findings demonstrate that Trx1 protein is necessary for the hypoxia-induced proliferation of

PASMC. We speculate that Trx1 may represent a novel therapeutic target for the vascular remodeling that underlies PH.

Key Words: Pulmonary hypertension, proliferation, vascular remodeling, thioredoxin 1 protein, pulmonary arterial smooth muscle cells, siRNA knock-down transfection

Dedicated to my loving parents Leif and Mary Ann Nelin, who have given me their never-ending support in the pursuit of my dreams.

ACKNOWLEDGMENTS

This project would not have been possible without the help, knowledge, and support of my numerous mentors. I am grateful to Dr. Trent Tipple for allowing me to work in his lab at Nationwide Children's Hospital in Columbus, Ohio. He has been an incredible mentor, and I have learned so much through my experience working there. I am thankful for his insight and encouragement in my endeavors both in the lab and out of it.

I would also like to thank my advisor and mentor Dr. Nancy Rice for her help throughout my four years here at Western Kentucky University, and with my thesis work as well. I am grateful for all of your wisdom and advice! Also thank you to the other two members of my committee, Dr. Crawford and Dr. Motley, for your willingness to devote time to my project.

The funding for this project was provided by Internal Funds from the Research Institute at Nationwide Children's Hospital and by Grant Number K08HL093365 from the National Heart, Lung, and Blood Institute.

Finally, I would like to thank my friends, family, and fellow students for the impact that they have had on my life these past four years! I know I would not be where I am today without their support and help through the setbacks as well as the rewards of my journey.

VITA

November 8, 1988Born – Alexandria, Louisiana

2007Olentangy High School, Lewis Center,
Ohio

2007Research Assistant for Dr. Trent
Tipple, Nationwide Children’s Hospital,
Columbus, OH

2007Academic Award of Excellence,
Western Kentucky University, Bowling
Green, KY

2007National/International Scholar,
Western Kentucky University, Bowling
Green, KY

FIELDS OF STUDY

Major Field: Biology

Minor Field: Chemistry

Emphasis: Pre-medicine

TABLE OF CONTENTS

	<u>Page</u>
Abstract	ii
Dedication	iv
Acknowledgements	v
Vita	vi
List of Figures	viii
Chapters:	
1. Background	1
2. Materials & Methods	5
3. Results	9
4. Discussion	15
Bibliography	17

LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
1	Trx System	3
2	Trx1 protein expression levels	11
3	Trx2, TrxR1, and Txnip expression levels	12
4	siRNA transfection of hPASMC to knockdown Trx1 protein expression	13
5	Hypoxia-induced proliferation of hPASMC depends on Trx1	14

CHAPTER 1

BACKGROUND

Pulmonary hypertension (PH) is seen in both children and adults and is associated with a clinical course of progressive deterioration interspersed with episodes of acute decompensation (Gaile, 2009). There is no cure for pulmonary hypertension (Ferns, 2009). The 5 year survival rate in patients with idiopathic pulmonary arterial hypertension treated with the vasodilator epoprostenol has been reported to be approximately 50% (McLaughlin, 2002). In children the outcomes may be even worse, for patients with chronic neonatal lung disease and PH the 2 year survival is only 25%, while the 2 year survival for patients with chronic neonatal lung disease and no evidence of PH is about 80% (Khemani, 2007).

It is now recognized that vascular proliferation and remodeling are the hallmark of PH (Morrell, 2009). The process of pulmonary vascular remodeling involves all layers of the vessel wall, and smooth muscle cells have a central role in vascular remodeling (Morrell, 2009). The proliferation and remodeling of the cell layers in the walls of the pulmonary arteries cause the lumens of the vessels to decrease in size. This causes an increase in blood pressure within these vessels and therefore the right side of the heart also increases in size because it must work harder to pump blood throughout the pulmonary circuit. Currently available therapies for PH treat only the symptoms of PH. The available therapies include vasodilator therapies which dilate the blood vessels but do not reverse pulmonary vascular remodeling (Ferns, 2009). There have been

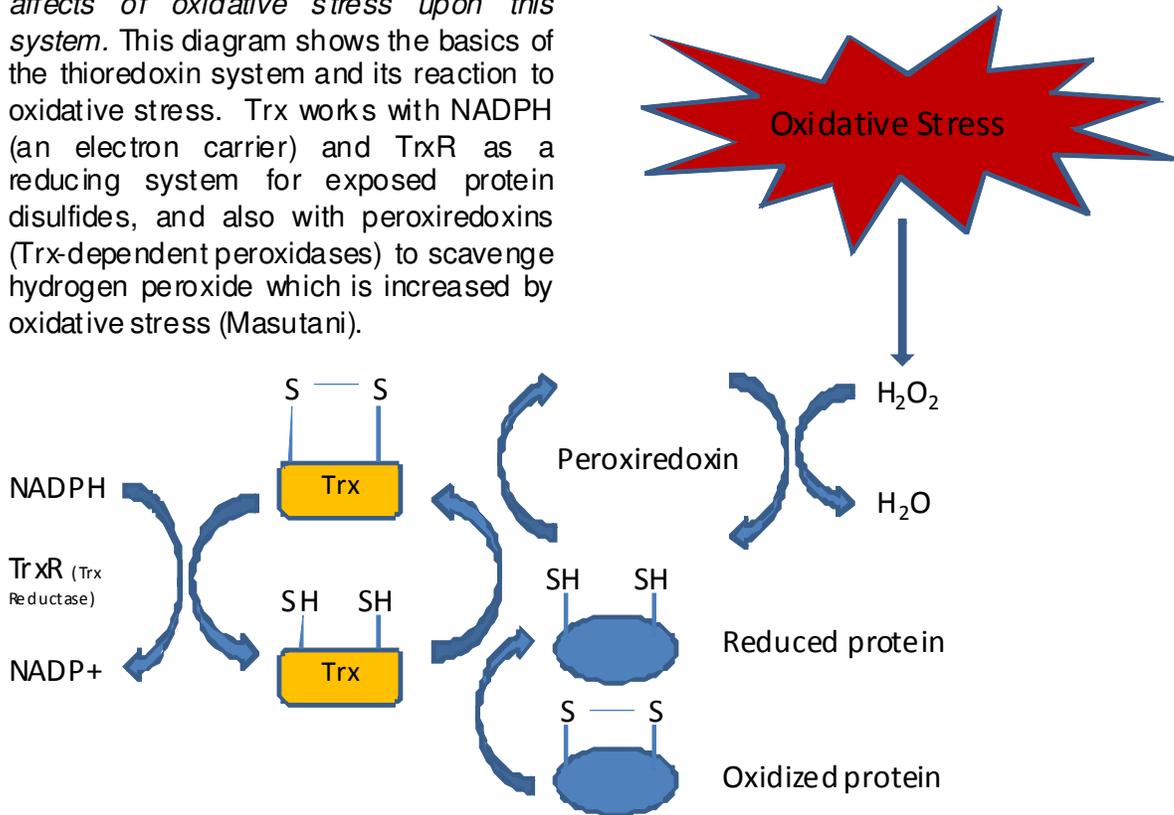
improvements in patient outcomes, although there are no curative therapies (Ghofrani, 2009). Often the success of therapy is measured in improvements in the distance walked in 6 minutes (the 6 minute walk test). Thus, new therapies and/or novel therapeutic approaches are desperately needed for this devastating disease.

Oxidative stress also has an important role in the development and the advance of PH. Recent data implicated oxidative stress as a mediator of PH and of the associated pathological changes to the pulmonary vasculature (DeMarco, 2010). Oxidative stress occurs when external insults cause excess reactive oxygen species (ROS) formation which overwhelms the antioxidant systems and creates an imbalance in the redox state of the cell, favoring oxidation. Excess ROS synthesis results in cell and tissue damage. Oxidative stress can contribute significantly to the pathogenesis of PH (DeMarco, 2010). Because ROS may promote vascular remodeling and smooth muscle cell proliferation, they are likely to play a significant role in many forms of PH (DeMarco, 2010). There are various types of ROS which are correlated with cardiovascular diseases, including superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2). ROS are harmful to subcellular organelles, like proteins, lipids, carbohydrates, and DNA, and thus a system to limit their action is necessary for cell survival, the antioxidant system. An example of an antioxidant system is the thioredoxin system, which acts as a thiol-reducing system (Park, 2010).

Thioredoxin (Trx), a ubiquitously expressed 12 kDa protein that along with thioredoxin reductase (TrxR) constitutes the Trx system, which is important in maintaining the oxidative balance inside of the cell. There are 2 isoforms of Trx, Trx1 is the predominant form of the thioredoxin protein, and Trx2 which is an 18 kDa protein that contains a 60 residue N-terminal sequence for targeting to the mitochondria (Collet, 2010). TrxR reduces oxidized Trx so that Trx can act as an electron donor to

peroxidases and ribonucleotide reductases in the cell, which then eliminate ROS in the cell (Park, 2010). However, Trx tends to exert most of its ROS-scavenging properties through Trx peroxidase, which is reduced by Trx1 and can scavenge ROS, like H_2O_2 by breaking it down into water and alcohol (Yamawaki, 2003). Thus, thioredoxins are essential for cell life in mammals. Thioredoxin interacting protein (Txnip) is another component of the Trx system, and acts as a negative regulator of Trx function, inhibiting its activity by interacting with the catalytic site of Trx (World, 2006). There is also evidence that Txnip has a role in cardiovascular disorders, like PH, by functioning as a sensor for oxidative stress (World, 2006).

Figure1. *Thioredoxin system and the affects of oxidative stress upon this system.* This diagram shows the basics of the thioredoxin system and its reaction to oxidative stress. Trx works with NADPH (an electron carrier) and TrxR as a reducing system for exposed protein disulfides, and also with peroxiredoxins (Trx-dependent peroxidases) to scavenge hydrogen peroxide which is increased by oxidative stress (Masutani).



PH is defined by a mean pulmonary artery pressure (PAP) in excess of 25 mmHg at rest (DeMarco, 2010). Hypoxia induces an immediate increase in PAP, and initiates and sustains an inflammatory response over time. Because of these changes, especially the increase in the number of inflammatory cells, there is also an increase in ROS during hypoxia (DeMarco, 2010). Thus, hypoxia can be used as an ideal model of PH.

Trx1 expression in some cells types can be augmented following hypoxic exposure. For example, Trx1 expression is shown to significantly increase in response to hypoxia exposure in rat hippocampal neurons (Stroev, 2009). Trx1 expression has also been shown to be elevated in response to hypoxia in human lung cancer tissues (Kim, 2003). Trx1 is also known to have a direct growth stimulating activity (Berggren, 1996) and proliferation increases as a result of Trx1 secretion. The role of Trx1 in hypoxia-induced proliferation and remodeling in PASMC has not been studied. Therefore, we hypothesized that as a result of hypoxia; Trx1 protein expression in PASMC will increase, and as a result, so will the proliferation of these cells.

RNA interference (RNAi) is a process to regulate gene expression within the cell. In order to suppress gene expression, a RNAi gene-silencing technique uses small-interfering RNA (siRNA). A specific siRNA will target a specific mRNA sequence within the cell, and is directed to this target RNA by an RNAi induced silencing complex (RISC). The antisense strand of the siRNA, along with RISC, directs the degradation of the complementary mRNA. One of the most common ways in which siRNA is introduced to the cell is through transfection. Transfection is conducted following a specific protocol, which includes using a scramble siRNA as a positive control. The scramble siRNA is made up of the same nucleotides as the siRNA, however, they are arranged in a different order which does not affect expression of the target mRNA.

CHAPTER 2

MATERIALS AND METHODS

Pulmonary arterial smooth muscle cell (PASMC) culture: Human PASMC (Lonza Group Ltd, Switzerland) are grown in 21% O₂, 5% CO₂, balance N₂ at 37°C in smooth muscle growth media (SmGM, Lonza), which includes 5% fetal bovine serum, 0.5 ng/ml human recombinant epidermal growth factor, 2 ng/ml human recombinant fibroblast growth factor, 5 µg/ml insulin and 50 µg/ml of gentamicin. The hPASMC are used in experiments between the fifth and eighth passages, throughout which no changes in cell morphology are noted. The cells are grown to ~80-90% confluence, washed, fresh media is placed on the cells, and they are incubated in 21% O₂, 5% CO₂, balance N₂ (normoxia) or 1% O₂, 5% CO₂, balance N₂ (hypoxia) for 24, 48, 72, or 120 hours.

Protein isolation. Protein is isolated from hPASMC as previously described (Nelin, 2001). Briefly, hPASMC are washed with phosphate-buffered saline (PBS), and 50-100 µl of lysis buffer (0.2M NaOH, 0.2% SDS) is added to each plate or each well of a six-well plate. 30 minutes before use, the following protease inhibitors are added to each ml of lysis buffer: 1µl aprotinin [10 mg/ml double distilled (dd) H₂O], 1µl leupeptin (10 mg/ml ddH₂O), and 1µl of phenylmethylsulfonyl fluoride (34.8 mg/ml methanol). The lysis buffer solution is added to each plate or each well of a six-well plate. The hPASMC are scraped, pipetted into sterile centrifuge tubes, and placed on ice for 30 minutes. The cell lysates are centrifuged at 12,000 x g for 10 minutes. The supernatant is stored in 1.5

ml tubes at -80°C. Total protein concentration is determined by the Bradford method (Bio-Rad, Hercules, CA).

Western Blot. The lysed hPASCs are assayed for Trx1, Trx2, TrxR and Txnip protein levels by western blot analysis as previously described (Chen, 2009). Aliquots of cell lysate are diluted 1:1 with SDS sample buffer, heated to 80°C for 15 minutes, and then centrifuged at 10,000 x *g* at room temperature for 2 minutes. Aliquots of the supernatant are used for SDS-polyacrylamide gel electrophoresis. The proteins are transferred to polyvinylidene difluoride membranes and blocked for two hours in PBS with 0.1% Tween (PBS-T) containing 10% nonfat dried milk. The membranes are then incubated with primary antibody, Trx1, Trx2, TrxR or Txnip, overnight and then washed three times with PBS-T. The membranes are incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (1:10,000, Bio-Rad) for 1h, then washed 3 times with PBS-T. The protein bands are visualized using enhanced chemiluminescence (ECL plus reagent; Amersham Pharmacia Biotech, Piscataway, NJ) and quantified using densitometry (Sigma Gel, Jandel Scientific, San Rafael, CA). To control for protein loading, the blots are stripped using a stripping buffer containing 62.5 mM Tris HCl (pH 6.8), 2% SDS, and 100 mM 2-β-mercaptoethanol, and the blots are reprobbed for β-actin (1:10,000; Abcam, Cambridge, MA).

Proliferation Assay. To determine cell proliferation, hPASCs (Lonza) are seeded in 6-well plates (~10,000 cells per well) in SmGM and incubated in normoxia or hypoxia for 120 hours. Adherent cells are trypsinized, and viable cells counted using a hemacytometer and trypan blue exclusion.

Transfection. To determine the effects of Trx1 knock-down on hPASC proliferation, transient transfection of the siRNA for the Trx1 gene was performed with

DharmaFECT 1 transfection reagent (Thermo Fisher Scientific, Lafayette, CO) according to manufacturer's protocol. Briefly, in two 1.5 ml centrifuge tubes, 17.5 μ L of 2 μ M Trx1 siRNA or scramble siRNA, was mixed with 17.5 μ L of serum-free smooth muscle basal medium (Lonza), vortexed and incubated at room temperature for 5 minutes. In two separate 1.5 ml centrifuge tubes, 1.4 μ L of the DharmaFECT transfection reagent was mixed with 33.6 μ L of serum-free smooth muscle basal medium (Lonza), vortexed and incubated at room temperature for 5 minutes. One of the transfection reagent tubes was then added to the each tube of the siRNA mixture, mixed, and incubated at room temperature for 20 minutes. SmGM (280 μ L) was then added to each tube for a total volume of 350 μ L. For the vehicle-treated hPASMCM, ddH₂O was used in place of siRNA.

Statistical Analysis. The data are shown as mean \pm standard error. The protein levels for each protein of interest were compared for effects of time and oxygen tension using a two-way analysis of variance (ANOVA). The protein levels for normoxic and hypoxic values were compared at individual time points using a t-test. The proliferation studies were compared using a one-way ANOVA with a student Newman Keuls post-hoc test to identify group differences. Differences were considered significant when $p < 0.05$.

Experimental Protocols: In the first set of studies, the expression levels of Trx1, Trx2, TrxR and Txnip proteins in PASMCM cultured in either normoxia or hypoxia were determined. The PASMCM were grown for 24, 48, 72, and 120 hours as described above, and incubated in either normoxia or hypoxia. At each time point, protein was harvested and used in Western blotting analysis for Trx1, Trx2, TrxR, and Txnip protein levels. Experiments were repeated 4-6 times for statistical analysis.

In a second set of experiments, PASMCM were used in proliferation assays as described above. The PASMCM were transfected with either a Trx1 siRNA construct or a scramble siRNA construct as described above, and placed in either normoxia or

hypoxia, such that there were 6 groups, normoxic vehicle treated, normoxic Trx1 siRNA treated, normoxic scramble siRNA treated, hypoxic vehicle treated, hypoxic Trx1 siRNA treated and hypoxic scramble siRNA treated. After 120 hours the PASMNC were harvested and counted as described above. Some of the PASMNC from the wells are harvested for protein so that Western blot analysis can be done to confirm knock-down of Trx1. Experiments were repeated 4-6 times for statistical analysis.

CHAPTER 3

RESULTS

The effect of hypoxia on the expression of the thioredoxin proteins. The hPASMC were exposed to either hypoxia or normoxia for various times (24 hours, 48 hours, 72 hours, and 120 hours). At each time point the protein was harvested and stored in the -80°C freezer. The protein concentration of the cell lysates was then determined and samples for Western blotting made. Western blot analysis was conducted with primary antibodies against Trx1, Trx2, Txnip, and TrxR. We found that the expression of Trx1 protein was significantly ($p < 0.001$) higher after 72 hours of hypoxia than in cells exposed to normoxia for 72 hours; the expression of Trx1 in hypoxia appeared higher than in normoxia after 48 hours as well, however this difference was not significant ($p = 0.06$) (Figure 2). This trend may become significant if more experiments were done. By a two-way ANOVA, we determined that for Trx1 there was interaction between the time of exposure and the oxygen tension of the exposure ($p < 0.001$) (Figure 2). We also found that Trx2, TrxR and Txnip are all expressed in the hPASMC (Figure 3). However, hypoxia had little effect on the protein levels of Trx2, TrxR or Txnip and there was no interaction between the time of exposure and the oxygen tension of the exposure (Figure 3).

Expression of Trx1 protein is prevented by the siRNA transfection in normoxia and hypoxia. We found that the transfection of the hPASMC with the siRNA against Trx1 was successful in knocking down Trx1 protein expression (Figure 4). There were

no discernable bands on the Trx1 Western blot in lanes containing the Trx1 siRNA treated hPASC (lanes 1-4) and easily discernable bands on the β -actin Western blot in the same lanes, thus demonstrating the knockdown of Trx1 protein expression. Lanes 5-8 showed the expression of Trx1 in hPASC treated with scramble siRNA, which is a construct that has the same nucleotide bases but is arranged in a different order. This scramble siRNA functions as a control. Lanes 9-12 represent the hPASC vehicle treated samples, which are treated with ddH₂O instead of an siRNA construct. This figure not only shows Trx1 knockdown, but also reaffirms the fact that Trx1 protein expression is higher in hypoxia than in normoxia. By observing the difference in band intensity of the control lanes, it can be seen that lanes 9 and 10 (hypoxia-exposed) are much darker than lanes 11 and 12 (normoxia-exposed). This means that Trx1 was expressed at a higher level in the samples exposed to hypoxia than those exposed to normoxia.

Hypoxia-induced proliferation depends on Trx1 protein. Our proliferation studies revealed that Trx1 is an important factor in the hypoxia-induced proliferation of hPASC (Figure 5). After the knockdown of Trx1 with the siRNA construct, we determined the proliferation of these transfected hPASC in both normoxia and hypoxia by counting the viable hPASC in each well of a 6-well plate. We found that the proliferation of hPASC treated with the siRNA targeting Trx1 was the same in hypoxia as it was in normoxia. Whereas in our experiments with scramble hPASC, we found the proliferation to be much higher in hypoxia than in normoxia. In the cells treated with the scramble siRNA construct the proliferation of hPASC was significantly higher in hypoxia ($p < 0.001$) than in normoxia. Also in both exposure groups, hypoxia and normoxia, the proliferation of the cells treated with the siRNA targeting Trx1 was significantly less than that of the cells treated with the scramble siRNA ($p < 0.01$).

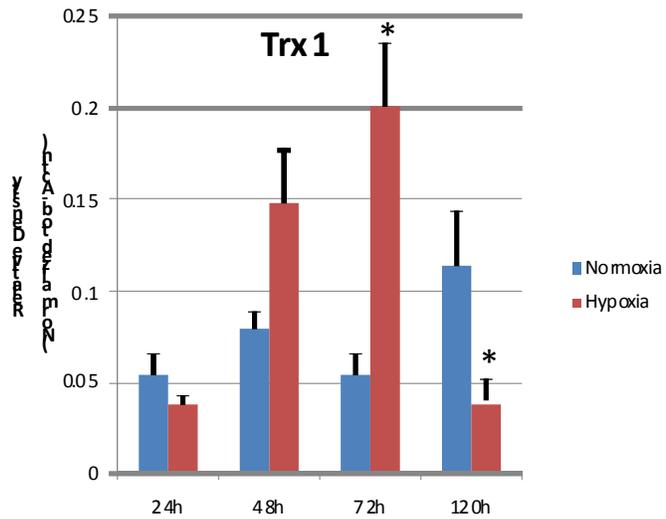


Figure 2. *Thioredoxin 1 protein expression in hypoxia is significantly higher than in normoxia at 48 hours and 72 hours.* hPASMC were exposed to hypoxia and normoxia over specified periods of time. They were then assayed with a primary antibody against Trx1 to determine the level of expression of the Trx1 protein in the hPASMC. * Trx1 expression in hypoxia different than normoxia at same time point ($p < 0.05$).

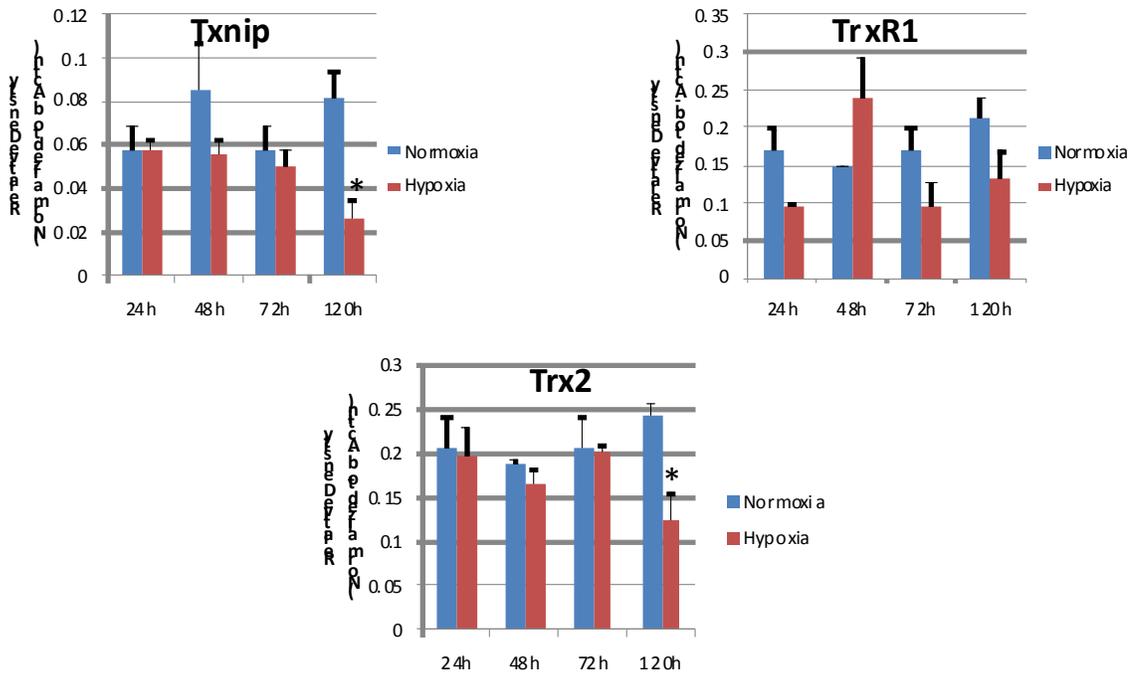


Figure 3. *Trx2*, *TrxR1*, and *Txnip* expression levels are largely unaffected by exposure to hypoxia. hPASCs exposed to hypoxia and normoxia over specified periods of time were assayed with primary antibodies against *Trx2*, *TrxR*, and *Txnip*. The expression levels of these proteins were not significantly different in the two conditions. * expression in hypoxia different from normoxia at the same time point ($p < 0.05$).

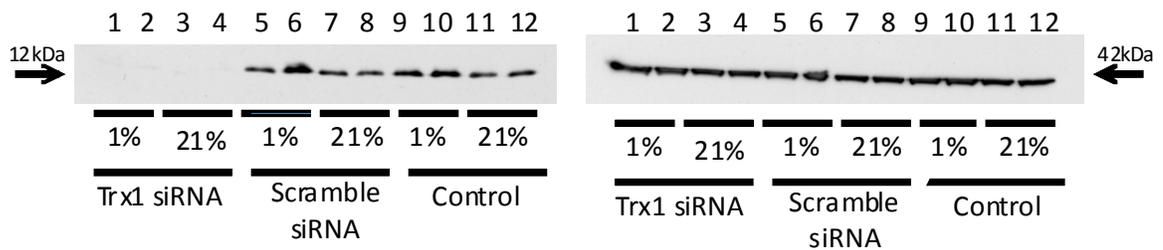


Figure 4. *siRNA transfection of hPASCs with siRNA targeting Trx1 effectively knocks down Trx1 protein expression.* hPASCs were transfected with a siRNA targeting Trx1 and then exposed to hypoxia or normoxia for 120 hours. The controls were samples of hPASCs treated with a scramble siRNA and hPASCs treated with no siRNA. Trx1 expression was effectively silenced by the siRNA transfection, shown by the disappearance of bands 1-4 in the Trx1 (12 kDa) Western Blot assay but not in the β -actin (42 kDa) assay. This figure again demonstrates that Trx1 expression is higher in hypoxia (1%) than in normoxia (21%). On the Trx1-assayed blot, the band intensity is much higher in the hypoxia-exposed control samples (lanes 9 and 10) than in the normoxia-exposed control samples (lanes 11 and 12). (Control samples are simply hPASCs treated with transfection reagent and ddH₂O)

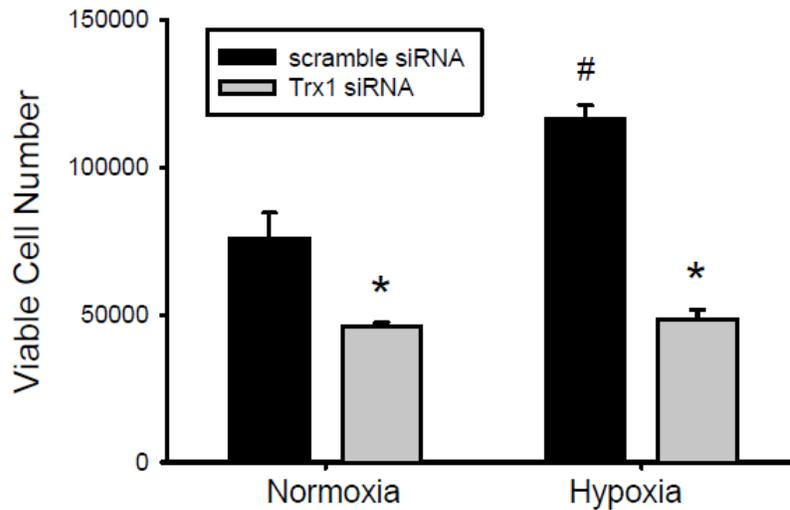


Figure 5. Hypoxia-induced proliferation of hPASMC depends on Trx1. hPASMC were transfected with either a scramble siRNA or an siRNA targeting Trx1. After 24 hours the cells were washed and allowed to recover for 24 hours. The cells were then plated into the wells of a 6 well plate at a density of 10,000 cells per well and 5 days later cell numbers were determined using trypan blue exclusion. * Trx1 siRNA different from scramble siRNA same condition, $p < 0.01$. # hypoxia different from normoxia same treatment, $p < 0.001$. hPASMC proliferation is significantly higher in hypoxia compared to normoxia, however after transfection with Trx1 siRNA (knockdown of gene expression), the proliferation of hPASMC is the same in both normoxia and hypoxia.

CHAPTER 4

DISCUSSION

The main findings from this study were that in hPASC: 1) hypoxia increased the expression of Trx1 protein, 2) Trx1 protein levels could be knocked-down using an siRNA, 3) hypoxia increased proliferation of hPASC and 4) knock-down of Trx1 protein prevented hypoxia-induced proliferation in hPASC. Our results confirm our hypothesis that Trx1 protein expression is induced in hPASC by exposure to hypoxia and that Trx1 protein expression is necessary for hypoxia-induced proliferation in hPASC. This discovery is important because by regulating the expression of Trx1, we may be able to regulate the proliferation of hPASC in a model of pulmonary hypertension.

Following exposure to hypoxia, hPASC expression of Trx1 protein is increased. Normal Trx1 expression is key in maintaining redox balance within these cells. Following certain signals, Trx1 is reduced by TrxR and thus can act as an electron donor to peroxiredoxin, which acts as a ROS scavenger reducing harmful H₂O₂ to water within the cell. Because hypoxia is a model for PH, we can conclude that during the course of PH, at least initially, Trx1 protein expression is increased and the redox balance of the cell is disturbed. The increase in expression of Trx1 is likely due to the increase of ROS within the cell due to hypoxia exposure. It is the hPASC's way of trying to decrease the number of ROS, and return to a balanced redox state.

Through transfection using an siRNA against the Trx1 protein, we were able to successfully knock-down the protein levels of Trx1. siRNA binds to the site on the cell's

mRNA where Trx1 protein translation occurs, and cuts the mRNA which prevents Trx1 protein translation. By doing this, the role of Trx1 protein within the hPASMC's can be studied. By observing changes in hPASMC behavior in the absence of Trx1, we can determine what cellular activities it is an important part of. That was our reasoning for knocking-down its expression in this study, to observe its role in hypoxia-induced proliferation of hPASMC.

Exposure to hypoxia increased the proliferation of hPASMC. This is consistent with the development of PH in the lung. This increased proliferation of hPASMC within the vessels is what causes the lumen of pulmonary arteries to decrease in size, and blood pressure in the lung to increase. It is important to determine why hypoxia exposure leads to the proliferation of hPASMC.

The successful knock-down of Trx1 protein prevented hypoxia-induced proliferation in hPASMC. This finding confirms our postulate that Trx1 protein has an important role in hPASMC proliferation. In the hPASMC with no Trx1 protein, the proliferation of the hPASMC in hypoxia remained the same as those in normoxia. However, in the control group (scramble siRNA), the proliferation of hPASMC in hypoxia increased compared to those in normoxia. We speculate that Trx1 protein may be important in regulating the cell cycle, perhaps having a role in controlling checkpoint proteins which regulate progression through the cell cycle. Alternatively, Trx1 protein levels may be important in preventing apoptosis, such that in the cells with knock-down of Trx1 there is increased apoptosis and therefore decreased viable cell numbers. Future studies will examine the mechanism(s) underlying the positive effect of Trx1 on hypoxia-induced proliferation of hPASMC.

BIBLIOGRAPHY

1. Berggren M, Gallegos A, Gasdaska JR, Gasdaska PY, Warneke J, Powis G. *Anticancer Res* 16(6B):3459-66, 1996.
2. Chen B, Calvert AE, Cui H, Nelin LD. *Am J Physiol Lung Cell Mol Physiol* 297:L1151-9, 2009
3. Collet JF, Messens J. *Antioxid Redox Signal* 13(8):1205-1216, 2010.
4. DeMarco VG, Whaley-Connell AT, Sowers JR, Habibi J, Dellsperger K. *World J Cardiol* 2(10):316-324, 2010.
5. Ferns SJ, Wehrmacher WH, and Serratto M. *Comprehensive therapy* 35:81-90, 2009.
6. Gaile MMH, M. Humbert, A. Torbicki, J-L. et al. *Eur Respir J* In Press: 2009.
7. Ghofrani HA, Barst RJ, Benza RL, et al. *J Am Coll Cardiol* 54:S108-117, 2009.
8. Khemani E, McElhinney DB, Rhein L, et al. *Pediatrics* 120:1260-1269, 2007.
9. Kim HJ, Chae HZ, Kim YJ, Kim YH, Hwangs TS, Park EM, Park YM. *Cell Biol Toxicol* 19(5):285-98, 2003.
10. Masutani H, Ueda S, Yodoi J. *Cell Death and Differentiation* 12:991-998, 2005.
11. McLaughlin VV, Shillington A, Rich S. *Circulation* 106:1477-82, 2002
12. Morrell NW, Adnot S, Archer SL, et al. *J Am Coll Cardiol* 54:S20-31, 2009.
13. Nelin LD, Nash HE, Chicoine LG. *Am J Physiol Lung Cell Mol Physiol* 281:L1232-L1239, 2001.
14. Park KJ, Kim YJ, Choi EJ, Park NK, Kim GH, Kim SM, Lee SY, Bae JW, Hwang

KK, Kim DW, Cho MC. *Korean Circ J* 40(12):651-658, 2010.

15. Stroeve SA, Tyul'kova EI, Glushchenko TS, Tugoi IA, Samoilov MO, Pelto-Huikko

M. *Neurosci Behav Physiol* 39(1):1-5, 2009.

16. World CJ, Yamawaki H, Berk BC. *J Mol Med* 84:997-1003, 2006.

17. Yamawaki H, Haendeler J, Berk BC. *Circulation Research* 93:1029-1033, 2003.