INTRODUCTION

Vasoreactivity is an important characteristic of vessels in the body as it allows the body to compensate for various changes in the environment. The vascular smooth muscle cells (VSMCs) in the body provide tone to the vessels which allow them to contract as relax as needed. VSMCs exist in two different phenotypes in the body, synthetic and contractile. In the contractile state, the cells are non-proliferative and react to stimuli to contract and relax the vessels, while in the synthetic state the cells are proliferative and do not serve the function of contracting and relaxing vessels.

Halayko et. al [1] described that a vascular smooth muscle cell has both phenotypic and mechanical plasticity. The phenotypic plasticity is seen in the expression of various markers such as smooth muscle alpha actin (SMαA), myosin heavy chain (myh11), and transgelin (sm22), which play important physiological roles in arranging the contractile apparatus of VSMCs.

Although the synthetic phenotype of SMCs is considered the disease state, it is occasionally beneficial for the cells to have proliferative properties to help the body recover from injury. Previous literature has shown that using serum starvation, or the removal of serum from the media of the cells, can induce phenotypic switching in smooth muscle cells [2]. Removing the serum from the media causes a biochemical change in the cell causing it to switch from the synthetic to the contractile phenotype. This change can be seen when looking at smooth muscle cells markers as they are upregulated in the contractile state. The ability to harness the state in which the cells exist is essential to seeing the effects of various stimuli to contract and relax the vessels.

During a stroke, the brain is deprived of oxygen for extended periods of time often resulting in the death of neurons and decreased mental acuity. Currently, stroke affects approximately 795,000 Americans each year [3] and is the leading cause of disability in the United States [4]. The approach to rehabilitation involves coupling exercising the muscle that was involved in the brain region affected by the stroke with pain medication for discomfort. To increase efficacy of rehabilitation, smooth muscle cells can be stimulated to proliferate and create new vessels in the brain to allow increased oxygen delivery to the affected area.

Previous evidence from the Straub lab has shown that cytochrome b5 reductase 3 (Cyb5R3) controls cyclic guanosine monophosphate (cGMP) levels by regulating the redox state of soluble guanylate cyclase (sGC). It is known that cGMP regulates protein kinase G, which is important for keeping the VSMCs in a contractile state. Using this information, we will be investigating the role of Cyb5R3 in the phenotypic modulation of VSMCs to further understand the and harness regulation of VSMC phenotype in various disease states.

OBJECTIVE

The objective of this study was to determine if Cyb5R3 can control the phenotypic switching of smooth muscle cells.

HYPOTHESIS/SUCCESS CRITERIA

Cyb5R3 does control phenotypic switching of smooth cells and its expression causes the cells to prefer the contractile phenotype.

If the change in mRNA levels between the various treatments is significant, then Cyb5R3 will be decided to have a role in phenotypic switching of smooth muscle cells.

METHOD

This study used non-targeting (NT), Cyb5R3 knockdown (KD), and naive rat aortic smooth muscle cells (raSMCs) to test the effect of Cyb5R3 in phenotypic switching. The KD and NT cells were plated in 2 separate 6-well plates and were allowed to grow to 90-100% confluence. The media that the cells were in was then changed from normal media to serum poor media, or serum starved, for 24, 48, and 72 hours.

Additionally, naive raSMCs were plated in a 6-well plate and one well was treated with GFP adenovirus, the second treated with Cyb5R3 overexpression virus, and the third was not treated with anything. These cells were serum starved for 72 hours.

All cells were harvested after their respective times in serum poor media using Trizol, and the RNA was isolated following the given extraction protocol.

The RNA was used to create cDNA using the SuperScript kit. Levels of Cyb5R3, SMαA, myh11, and sm22 mRNA were analyzed using qPCR. The qPCR was run in triplicate for each gene being analyzed and values of normalized mRNA were averaged to find mRNA expression levels as shown in Figures 1 and 2. The values were compared using a t-test and the results were considered significant if the p-value was less than 0.05.

RESULTS

As shown in Fig. 1, the mRNA levels of Cyb5R3, SMαA, myh11, and sm22 all increased after serum starvation induced phenotypic switching in the NT cells and had a p-value of less than 0.05, while there is no significant change in in mRNA levels in the KD cells (p>0.05).

When the cells were treated with virus and then serum starved, the cells infected with GFP did not show as robust of
an increase in SMaA, myh11, and sm22 when compared to the cells infected with the Cyb5R3 overexpression virus (Fig. 2). The cells showed a switch in phenotype which can be concluded from the increase in contractile markers.

When infected with adenovirus to overexpress Cyb5R3, the cells upregulated the contractile markers significantly more than when the cells were only infected with GFP (Fig. 2). The indication of phenotypic switch is more robust in the cells that overexpressed Cyb5R3, which shows that Cyb5R3 aids cells in switching from the synthetic to the contractile phenotype.

In the future, these same experiments will be applied to human brain vascular smooth muscle cells to see if they will elicit the same response as raSMCs. Additionally, a mouse model of stroke known as middle cerebral artery occlusion will be applied to mice to see changes in brain vasculature via immunofluorescent microscopy.

CONCLUSION
Cyb5R3 does control the phenotypic switching of smooth muscle cells and causes them to prefer the contractile state. Harnessing this switching using the level of expression Cyb5R3 in cells allows for the ability to control proliferation of these cells and the formation of new blood vessels in the brain after a stroke to increase efficiency and success of rehabilitation.

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REFERENCES

DISCUSSION
These results support the hypothesis that Cyb5R3 controls phenotypic switching of smooth muscle cells and makes cells prefer the contractile state. The increase in mRNA levels of SMaA, myh11, and sm22 in the NT cells, as shown in Fig 1, indicates that these cells are in fact switching from the synthetic state into the contractile state when induced via serum starvation. Seeing the increase in Cyb5R3 levels shows that the cells upregulate Cyb5R3 when in the contractile state. The KD cells did not show a significant change in mRNA levels of these smooth muscle cell markers demonstrating that decreasing mRNA levels of Cyb5R3 inhibits the cells ability to switch from the synthetic state to the proliferative state.

Figure 1. Plots of normalized mRNA levels of Cyb5R3 (top left), myh11 (top right), SMaA (bottom left), and sm22 (bottom right) in NT (white) and KD (red) cells after serum starvation for 24, 48, and 72 hours.

Figure 2. Plots of normalized mRNA levels of myh11 (left), SMaA (middle), and sm22 (right) of raSMCs treated with adenovirus and serum starved for 72 hours. Naïve raSMCs are shown in black. GFP infected cells are shown in gray. Cyb5R3 overexpression infected cells are shown in green.