INTRODUCTION

Bicuspid aortic valve disease (BAV) is the most common congenital cardiac malformation occurring in 1-2% of the general population [1] [2]. Normally, the aortic valve is separated into 3 valves. However, BAV involves fusing of the valvular leaflets during development, resulting in a 2-valve structure instead of the normal 3-valve structure. BAV predisposes patients to a thoracic aortic aneurysm, which is characterized by an enlargement of the ascending aortic vessel [3]. Causes of aneurysm can be attributed to an underlying weakness in the vessel walls. These aneurysms can be life threatening, particularly if they result in a ruptured vessel wall. It has been shown that patients with BAV disease typically present a thoracic aortic aneurysm up to 10-15 years earlier than patients with a normal tricuspid aortic valve (TAV) [3].

Prior to a thoracic aortic aneurysm, degeneration at the tissue level is prevalent. Degeneration can cause weakening of the aortic wall, which then can lead to aneurysm. While it is known that patients with BAV have increased risk of a thoracic aortic aneurysm, the cellular mechanisms remain unknown. Understanding the cellular mechanisms is important in studying pathological characteristics of the aorta, such as BAV [4].

Smooth muscle cells play a key role in tissue damage repair and could help to explain the degeneration of tissue prior to thoracic aortic aneurysms. The smooth muscle cells have remarkable plasticity and can exist in a synthetic phenotype, where the cells are continually growing and proliferating, or in a contractile phenotype, where the cells contribute to regulation of vessel tone [4]. Smooth muscle cells alter their phenotype based on environmental cues such as nutrients availability [5]. Fetal bovine serum is used as a growth factor when culturing cells in vitro that offers the cells enough nutrients to continually proliferate. Depending on the smooth muscle cells’ phenotype, the cells can express different proteins suited for that phenotype’s particular function. Proteins that are characteristic of the contractile phenotype include smooth muscle actin, calponin, and smoothelin [5].

Studying the cellular differences, specifically in smooth muscle cells, between patients with bicuspid and tricuspid aortic valves may indicate why the pathology of a bicuspid aortic valve can initiate thoracic aortic aneurysms earlier in these patients. Therefore, a look at the phenotype of the smooth muscle cells in the aorta when they are subject to different environmental cues, such as nutrient levels, is important.

OBJECTIVE

In order to study cell phenotype in the BAV and TAV patients when subject to different environmental factors, the aortic smooth muscle cells will be treated with various media conditions as they are cultured in vitro. After the cells have been cultured in the different condition, the gene expression of phenotype protein markers can be quantified. The aortic smooth muscle cells will come from both TAV and BAV patients with and without aneurysms.

HYPOTHESIS

It is hypothesized that the aortic smooth muscle cells from all patient groups will exhibit a more synthetic, proliferative phenotype when treated with the media conditions containing the growth factor fetal bovine serum. This shift toward a synthetic phenotype will be represented by a decrease in the amount of gene expression of contractile protein markers.

METHODS

Tissue samples were obtained from 4 patient groups: BAV ± aneurysm and TAV ± aneurysm. TAV patients without aneurysm represent the control group for this study. Smooth muscle cells from the tissue samples were cultured in 4 different media. These medias include a smooth muscle cell specific media that contains fetal bovine serum (SMC Media), media with 10% fetal bovine serum added (+10% FBS), media with 10% insulin, transferrin, and selenium added (+10% ITS), and finally a general cell media without fetal bovine serum (DMEM). These different medias were either used for 48 hours or 5 days. This results in a total of 8 different culture conditions for the smooth muscle cells (4 media conditions at 2 time points).

As the cells grow in culture they will express different proteins depending on whether they are in a synthetic or contractile phenotype. As cells synthesize proteins they first will transcript DNA to mRNA. This mRNA will then be translated into proteins. The mRNA from the cultured smooth muscle cells was isolated after the cells were treated with the different conditions. The amount of mRNA of a certain gene will indicate how much of that protein would be expressed. Therefore, after the mRNA was isolated from the smooth muscle cells, contractile phenotype markers (smooth muscle actin, calponin, and smoothelin) needed to be quantified to determine if there was more or less of this phenotype marker in the culture condition with FBS.

The technique used to quantify amounts of gene expression was quantitative Polymerase Chain Reaction (qPCR). This technique detects target genes in the mRNA through fluorescent probes. The levels of fluorescence can be quantified to indicate how much of that target gene is present in the mRNA. The qPCR experiments were operated in the Genomics Department of Bioengineering, University of Pittsburgh and Bicuspid aortic valve (BAV) disease is the most common congenital cardiac malformation occurring in 1-2% of the general population [1] [2]. Normally, the aortic valve is separated into 3 valves. However, BAV involves fusing of the valvular leaflets during development, resulting in a 2-valve structure instead of the normal 3-valve structure. BAV predisposes patients to a thoracic aortic aneurysm, which is characterized by an enlargement of the ascending aortic vessel [3]. Causes of aneurysm can be attributed to an underlying weakness in the vessel walls. These aneurysms can be life threatening, particularly if they result in a ruptured vessel wall. It has been shown that patients with BAV disease typically present a thoracic aortic aneurysm up to 10-15 years earlier than patients with a normal tricuspid aortic valve (TAV) [3].

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and Proteomics Core Laboratories at the University of Pittsburgh. To ensure equivalent amounts of mRNA were compared between samples, the mRNA was normalized to 10ng/µL for each sample. Additionally, gene expression levels were measured in relation to a “house-keeping” gene that had consistent expression in each sample. By analyzing the gene expression of contractile phenotype markers, it could be determined what culture conditions stimulated more contractile phenotypes.

The data collected was statistically analyzed using SPSS packaging and a Kruskal-Wallis nonparametric test. Then, a pairwise comparison could place the significance by having a p value less than 0.05.

RESULTS

Calponin and smooth muscle actin gene expression showed a trend that was higher in the culture conditions that did not contain the growth factor fetal bovine serum. This was consistent among the 4 patients groups. However, Smoothelin showed an opposite trend with lower expression in culture conditions that did not contain fetal bovine serum. Again, this was consistent among the 4 patient groups. Statistical analysis only showed significance in the smoothelin gene expression data for the 5-day conditions in the control patient group. The 48-hour treatments showed similar trends in smoothelin gene expression, yet lacked the statistical significance.

Figure 1. Relative gene expression of smoothelin after 5 days of culture conditions. Results are based from gene expression of a “house-keeping gene” that was consistent in all samples. The TAV noneurysmal group shows significantly less smoothelin expression in the DMEM and +10% ITS media conditions. Error bars on the graph represent standard error of the mean.

Smoothelin gene expression after 5 days was significantly lower in the culture conditions that did not contain the growth factor fetal bovine serum (p<0.05). This significance was only seen in the control group, but the other patient groups show a similar trend. The lower levels of gene expression in the DMEM and +10% ITS media indicate cells in these conditions without FBS growth factor were in a more synthetic phenotype.

DISCUSSION

Smooth muscle actin and calponin, while not having statistical significance, showed a trend that matched the hypothesis. There was more expression of these contractile markers in the culture conditions that did not contain FBS. However, the statistical significance remains with gene expression of smoothelin, which did not agree with our original hypothesis. Smoothelin, another contractile marker, showed less expression in the culture conditions that did not contain FBS. The FBS treatments were hypothesized to contain smooth muscle cells in a synthetic phenotype because the FBS offered nutrients for the cell to proliferate and grow. Smoothelin, however, was present in the culture conditions where we did not expect to see cells in a contractile phenotype.

Limitations to this study include the fact that smoothelin is specific to only smooth muscle cells, while smooth muscle cells as well as myofibroblasts could express the other protein markers. If some of the culture conditions allowed for growth of cells other than just smooth muscle cells, the data for smoothelin expression in those conditions could be skewed. Another limitation is that the in vitro cell cultures are not the same as physiologic conditions. Therefore it can only give an indication about the cellular activities in the body.

Looking to the future, additional phenotype markers could be analyzed for a more holistic view of the cellular activity. Also, cell morphology differs between the smooth muscle cell phenotype, so a morphological comparison could give additional indication of cell phenotype. By increasing knowledge of cellular activities in BAV patients, studies can move toward an explanation of the cellular mechanisms behind the tissue degeneration prior to aneurysms in an effort to decrease risks for BAV patients.

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