CHAPTER 1

INTRODUCTION

1.1 Background

Thoracic Aortic Aneurysm and Dissection (TAAD) is a disorder that involves the aorta, which is the large blood vessel that distributes the blood from the heart to the rest of the body. TAAD affects the upper part of the aorta, near the heart. This part of the aorta is called the thoracic aorta because it is located in the chest (thorax). In TAAD, the aorta can become weakened and stretched (aortic dilatation), causing a bulge in the blood vessel wall (an aneurysm). Stretching of the aorta may also lead to sudden tearing of the layers in the aortic wall (aortic dissection), allowing blood to flow abnormally between the layers. Aneurysm and dissection of the aorta may cause rupture and results in death of the patient.

TAAD is a dilatation of thoracic aorta with diameter of more than 1.5 times than that of normal regardless of the presence or absence of the dissection.[1] TAAD is in the top 20 leading cause of death in the USA and the rest of the world.[2] TAAD is very dangerous as 95% of the Thoracic Aortic Aneurysm (TAA) cases are asymptomatic[3] and having TAA increases the risk of developing dissection.[4] TAAD cases are often diagnosed when the aneurysm is already very large, when dissection has already occurred, or accidentally found when diagnosing other diseases.[4] TAAD is also associated with other diseases such as

Marfan syndrome, Loeys-Dietz syndrome and Ehler-Danlos syndrome, making TAAD inherited in familial pattern.[5,6]

Several known genes which are associated with TAAD have been recognized, including *TGFBR1, TGFBR2, MYH11, ACTA2, FBN1, MYLK, SMAD3* and two loci, *AAT1* and *AAT2*, has been identified though the genes involved are still unknown.[1,3,7]

Though the exact pathogenesis of TAAD is unclear, TGFβ pathway has been proposed to be heavily involved in formation of TAAD. In Marfan syndrome, mutation of FBN1 gene increases the release of TGF-β1 and correlates with TAA.[8-11] The TGFβ pathway can be activated via the canonical pathway and non-canonical pathway.

In the canonical pathway, TGFβ ligand attaches to TGFβRII and recruits TGFβRI to form a heterotetramer complex. The heterotetramer complex then phosphorylates SMAD2/3. A co-SMAD, SMAD4, binds to the phosphorylated SMAD2/3 and SMAD2/3/4 complex enters the nucleus and activates specific transcription factors.[12-15]

Other than the canonical pathway, TGFβ pathway can be activated several other pathways. Several known non-canonical pathways includes, Erk1/2,[16,17] JNK/p38,[16,18,19] PI3K/Akt,[16] RhoA/ROCK.[16,19] These canonical pathways can be SMAD-dependent or SMAD-independent.

The TGFβ pathway is a very tightly regulated and complicated process involving multiple pathways and myriad of signal transduction molecules controlled by phosphorylation of their kinase domains by protein kinases. Protein

kinases are phosphorylation enzymes. These enzymes transfer phosphate groups from high energy donor molecules such as ATP to specific substrate peptide, directing the peptides biological activity, localization and function.[20] Protein kinases are particularly prominent in signal transduction and coordination of complex pathways.

Though the method in diagnosing TAAD is already established clinically, the molecular profile of TAAD patient is not known. The microarray technology is chosen to analyze the molecular profile because it is able to analyze multiple peptides simultaneously, making it time saving and efficient method in analyzing molecular pathways.

Studying the profile of the phosphorylation of the kinase substrate peptides will be able to help understand the regulation of how the canonical or non-canonical pathway is activated after the control and mutant fibroblast is stimulated with TGFβ. In this study the kinase substrate peptide profile of TGFβ-stimulated fibroblast of TAAD patients with *TGFβR2* mutations will be analyzed using a commercially available Serine-Threonine Kinase Microarray chip.

1.2 Research Question

Which canonical and non-canonical pathways are activated in pathogenesis of TAAD, especially in patients with *TGFβR2* mutations?

1.3 Research Purpose

1.3.1 General Research Purposes

To study the profile of TGFβ-stimulated fibroblast using microarray technology on TAAD patients.

1.3.2 Specific Research Purposes

1. To study the potential of Kinase Substrate Microarray as an alternative method to screen TAAD patients.
2. To analyze how the canonical and non-canonical TFGβ pathway is regulated in TAAD patients.

1.4 Research Benefits

The benefits of this study are:

1. To give a better understanding about the involvement of the canonical and non-canonical TGFβ pathway in pathogenesis of TAAD, as microarray is able to detect multiple substrates simultaneously, so therapeutic prevention can be developed on the molecular level.
2. To learn and develop a potential alternative screening method for patients with TAAD.
3. To learn the possibility of a targeted therapy for TAAD.

1.5 Originality

This is the first study attempting to profile kinase substrate phosphorylation pattern on TAAD patients. No previous researches have been done associated with this study.