BIOMEDICAL SCIENCES / DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY

Analysis of Protein-Protein Interaction of ADAMTS7 with Cardiomyocytes using the Yeast Two-Hybrid System

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INTRODUCTION

Coronary artery disease (CAD) is one of the most prevalent diseases in the United States of America. About 20.1 million (7.2%) adults above the age of 20 years and above have CAD[1]. A disintegrin and metalloproteinase with thrombospondin motifs-7 (ADAMTS7 enzyme) is found to be associated with many inflammatory diseases such as CAD and atherosclerosis. Previous studies have shown ADAMTS7 enzyme interacts with the Cartilage oligomeric matrix protein(COMP) proteins present in the cardiac region and causes degradation of COMP protein which induces CAD in both knock-out and wild-type hyperlipidemic mouse models[2]. Also, the ADAMTS7 RNA expression is seen predominantly in the Heart muscles along with other areas including bone, cartilage, synovium, tendon, and ligament, all of which contain COMP and it was also detectable in meniscus, skeletal muscle, and fat tissue[3]. The study aims at determining *in vitro* domain interaction of ADAMTS7 enzyme with cardiomyocytes proteins causing CAD in humans using the Yeast 2 hybrid system (Y2H).

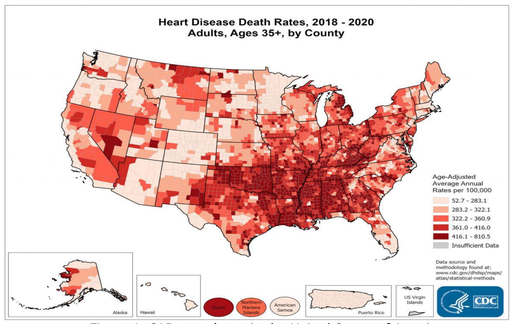


Figure 1. CAD prevalence in the United States of America.

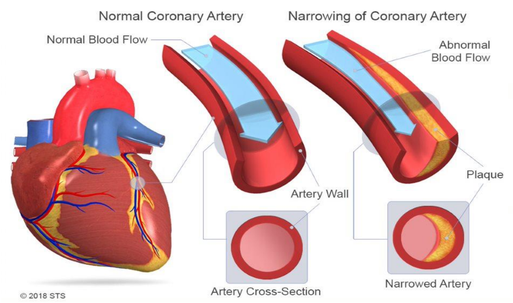


Figure 2. Formation of Coronary Artery Disease.

OBJECTIVES

- *To evaluate the association between the Adamts7 enzyme and CAD*

METHODS

Two yeast strains are grown, mated to generate a yeast 2 hybrid system used to determine the ADAMTS7 and CAD relationship.

- The ADAMTS7 encoding gene are cloned into the frame of bait plasmid and the prey plasmid generated using cDNA of c-*myc* gene from a CAD patient.
- Following, the bait and prey plasmids were transformed into Y2H Gold Yeast Strain and Y187 Yeast Strain vectors.
- Then, the bait plasmid are grown in dropout media to determine autoactivation.
- Extraction of the bait plasmid from Y2H Gold strain and colony polymerase chain reactions (PCR) done to ascertain ADAMTS7 presence.
- Y2H Gold and Y187 Yeast strains were incubated in the culturing tube for mating and zygote formation.
- The zygote formed were screened on QDO/X/A drop out media.

APPROACH

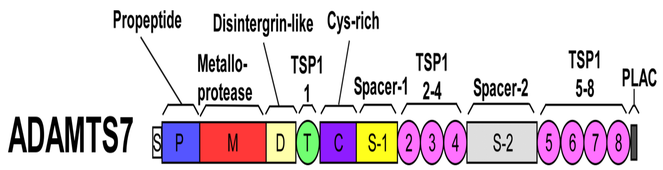


Figure 3. Domain structure of ADAMTS7

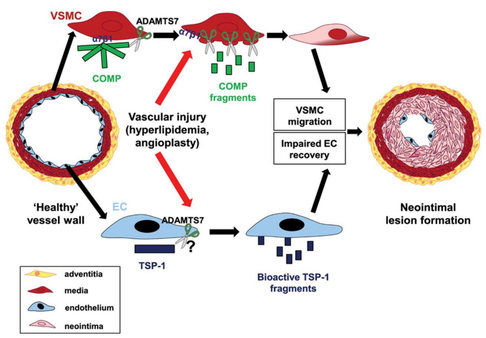


Figure 4. ADAMTS7-mediated actions on vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) promote neointima formation.

Figure 5. Yeast Transformation

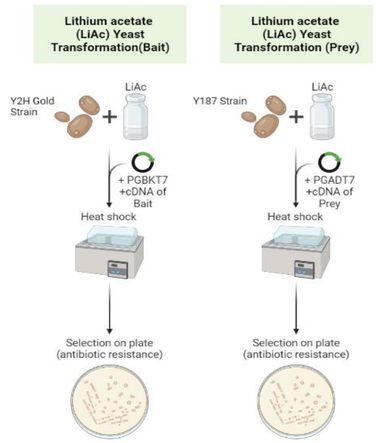


Figure 6. Autoactivation & Screening

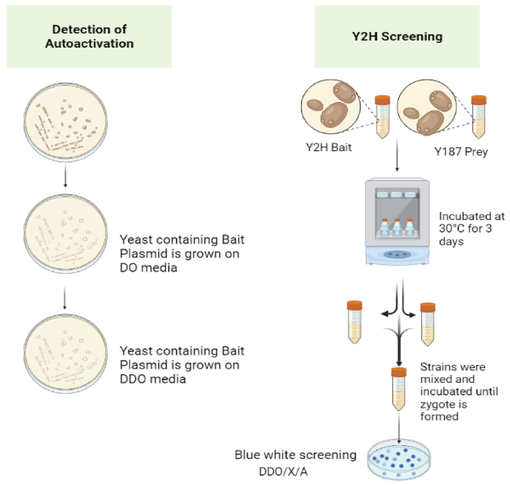


Figure 7. Plasmid Extraction

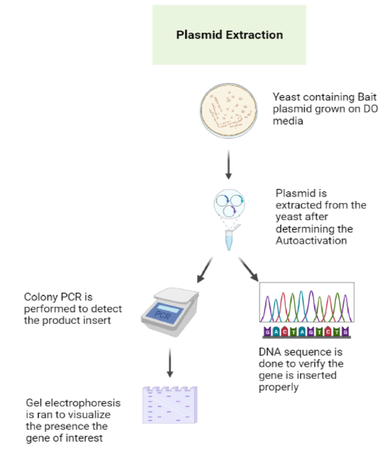
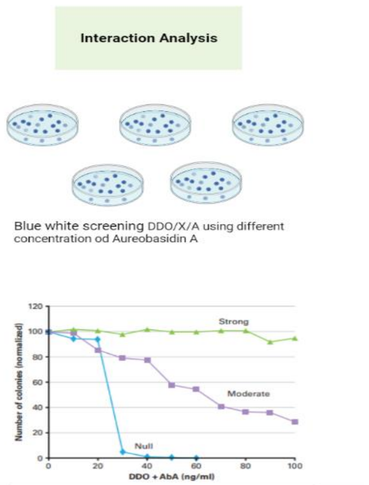


Figure 8. Interaction Analysis



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CONCLUSION

Yeast grown on DDO, DDO/X/A and QDO/X/A media and observed as blue or white colonies. Therefore, yeast containing interactive plasmids grew as blue colonies while the negative interaction colonies as white. The yeast strain employed contained a reporter gene whose regulatory regions contained DNA binding sites for protein X fusion (Bait) and the second hybrid protein Y fusion (Prey). Positive interaction between ADAMTS 7 and cardiomyocytes would result into bait and prey interactions activating upstream activating sequence (UAS). The interaction allows yeast to grow into blue colonies as it selectively expresses enzymes utilizing histidine amino acids. Therefore, if there is an ADAMTS7 enzyme interaction with cardiomyocytes then it confirms the relationship between ADAMTS7 and CAD.

REFERENCES

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3.Zhang, Y., J. Lin, and F. Wei, *The Function and Roles of ADAMTS-7 in Inflammatory Diseases.* Mediators Inflamm, 2015. **2015**: p. 801546.

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