Heart disease is the number one cause of death worldwide. Several predominant heart diseases are caused by atherosclerosis, the build-up of lipid-laden plaque, particularly cholesterol, blocking arteries. Atherosclerosis has been shown to be ameliorated by the process of reverse cholesterol transport mediated by plasma high-density lipoprotein (HDL, “good cholesterol”). HDL removes cholesterol from peripheral cells and transports it to the liver for conversion into bile and excretion. A major component of HDL is apolipoprotein A1 which interacts with the membrane-bound ATP-binding cassette transporter (ABCA1) to remove cholesterol from peripheral cells forming nascent HDL. The exact interactions between ABCA1 and apoA1 that produce nascent HDL are unknown and would be better understood by identifying the molecular structure of ABCA1. To simulate the native membrane lipid environment, lipid nanoparticles composed of a clipped ABCA1 containing the Rho (Rho) histidine (His), and rhodopsin (Rho). Transfection with the virus resulted in ABCA1 expression but the protein was quickly proteolyzed before it could be harvested, invalidating it as a possible tag for ABCA1 nanoparticle purification. In the future an ATP resin could be used as an alternative to express but the protein was quickly proteolyzed before it could be harvested, invalidating it as a possible tag for ABCA1 nanoparticle purification. The characterization of the Twin-Strap Tag thus aided in understanding the limits of modifications to the ABCA1 protein.

Introduction
- Atherosclerosis is the root cause of Coronary Artery Disease, the number one killer in the US.
- Atherosclerosis and plasma HDL levels are inversely related.
- The HDL-governed reverse cholesterol transport chain is largely responsible for the reduction in atherosclerosis.

Peripheral Cells
- ABCA1 interacts with apoA1 to create nascent HDL removing cholesterol from the peripheral cell.
- The structure of ABCA1 in a lipid membrane environment is unknown.
- Lipid nanoparticles are used to simulate the membrane environment to observe structure using cryo-electron microscopy.

Materials and Methods
- DNA Purification: To purify a plasmid containing a vector with target gene for bacmid creation.
- Recombinant Bacmid Transformation/Purification: Create an infectious bacmid containing target insert.
- Baculovirus Creation: Use a bacmid to create a baculovirus and transfet SF9 insect cells with ABCA1-His-Rho-Tag insert.
- Protein Purification: Determine presence of ABCA1 in transfected cells.

Results

Figure 1: Antibody Spot Test

Figure 2: Protein Purification with Rho Tag

Figure 3: Protein Purification with Twin-Strap Tag

Figure 4: Comparative Native Gel

Discussion
- Addition of TS tag in some way encouraged immediate post-transfection proteolysis of ABCA1.
- This resulted in the clipped ABCA1 seen in Figures 2-4.
- Antibody-binding site was likely proteolyzed removing the sequence the ABCA1 antibody binds to as seen in Figure 1.
- Some ABCA1 may not have been proteolyzed as seen in Figures 3 and 4.
- TS tag, despite being more efficient (Figure 4), is invalid as a purification method for ABCA1.

Future work will investigate the following alternatives:
- Since ABCA1 is an ATP-binding cassette transporter the validity of an ATP resin as an alternative resin is possible.

Conclusion
- Demonstrates that TS purification yields more concentrated protein pools.
- In combination with Figures 2 and 3 it reinforces that TS seemed to be a far more efficient purification method.

References

A. Since ABCA1 is an ATP-binding cassette transporter the validity of an ATP resin as an alternative resin is possible.

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