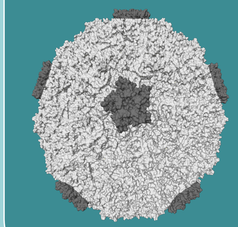
Examining the Effect of I100 of CDHR3 Solubility in *E. coli*

Andrew M. Lee, Soo Hyun Yang, and Jason McLellan
Freshmen Research Initiative Department at The University of Texas at Austin, 78712, TX

Abstract

Human rhinovirus is a virus that causes common cold illnesses. Rhinovirus C (RV-C), one of the three genetically distinct groups of rhinovirus, is mainly responsible for more severe illnesses such as asthma and pneumonia among children. On a molecular level, the expression of human protein, CDHR3, more specifically, its structural domain 1 and 2 are thought to be the binding sites for RV-C that is then introduced in our body. To determine the protein structure and its mechanism, purification can be done. However, CDHR3 is a transmembrane protein that remains in the cell debris due to its insolubility, preventing it from being purified. Furthermore, CDHR3 may not only bind to RV-C and have different functions. Therefore, site-directed mutagenesis was introduced on a target site of the gene and then its truncation mutant was created via Gibson Assembly to further purify and develop antibodies against the receptor to inhibit Rhinovirus infection.

Rhinovirus-C: The Most Frequent Causes of Respiratory Tract Infections



Human Rhinoviruses are small, nonenveloped, single-stranded, RNA viruses. Among the three main families (RV-A,B, and C), Rhinovirus-C has been the leading cause of respiratory tract infection among children as their primary host target in the airways remains unknown. Its current cure does not exist. Recent studies have shown that it has positive correlation with the expression of human transmembrane protein, CDHR3.

Figure 1. Structure of Human Rhinovirus-C (RV-C)

Association of Rhinovirus-C with Respiratory Infection Among Children

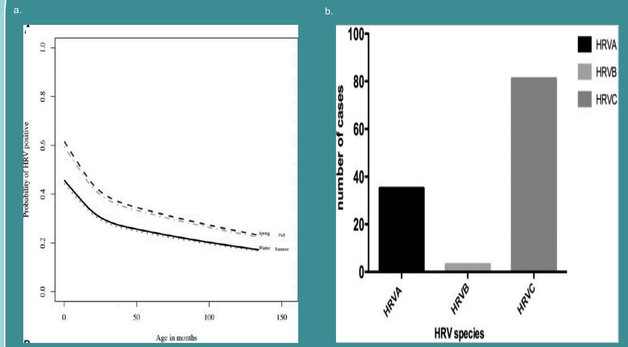
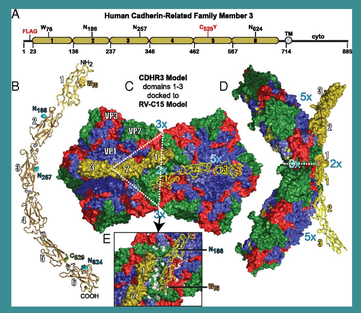


Figure 2. (a) Graph of Correlation between age in months and probability of HRV (Figure adapted from *Linder et al. 2012*) **(b) Frequency of HRV species detected at recruitment** (Figure adapted from *Cox et al. 2013)*

Rhinovirus-C (RV-C) binds to Cadherin-related family member 3 (CDHR3)



The expression of CDHR3, a human protein found in lung tissues, is proposed to be accountable for the binding of RV-C and its replication in human cells. The structure of CDHR3 consists of six domains to two of which the virus binds while the exact conformation is still unknown. During the protein purification process, CDHR3 remains in the cell debris as it is transmembrane protein and insoluble.

Figure 3. Structure Modeling of RV-C Binding to CDHR3
(Figure adapted from *Bochkov et al. 2015*).

Creating Truncation Mutant via Gibson Assembly

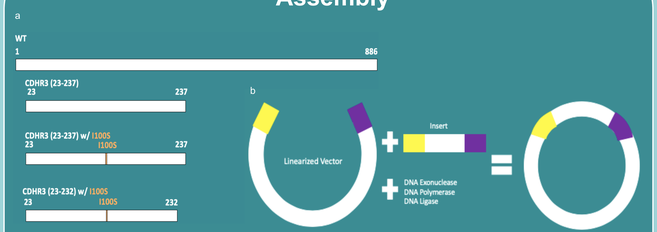
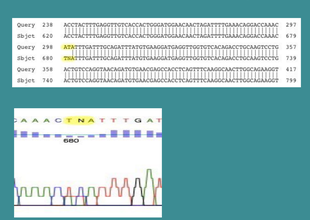


Figure 4. (a) Truncation Mutant (b) Gibson Assembly

Results

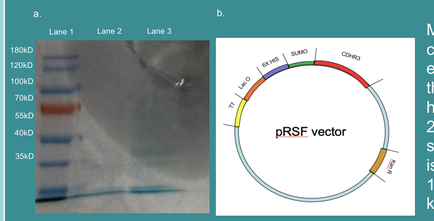
I100S Via Site-directed Mutagenesis



Site-directed mutagenesis was successfully introduced and confirmed through sequencing results as isoleucine (ATA) was mutated into serine (TCA). The DNA sequence trace showed that N amino acid was C with blue fluorescent dye.

Figure 5. (a) I100S sequence of CDHR3 (b) DNA sequence trace

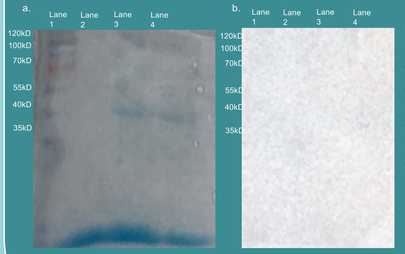
Optimal Expression Condition of BL21 Codon Plus



Mutant pRSF vector containing CDHR3 was expressed and confirmed through SDS PAGE. Well 1 had uninduced sample and 2 had induced sample. The size of the CDHR3 protein is 25kD and SUMO is 18kD, which equals to 43 kD shown in the image.

Figure 6. (a) Image of SDS PAGE gel of expressed protein (b) PRSF vector

Mutant CDHR3 Purification and Confirmation



Purified protein was run in SDS PAGE and analyzed with Western Blot to confirm the presence of CDHR3 protein. However, the protein was not shown neither in the gel nor in the transfer membrane.

Figure 7. Image of (a) SDS PAGE gel and (b) Western Blot

Conclusions and Future Direction

- I100S via site-directed mutagenesis was successfully introduced
- I100S was not enough to increase the solubility of CDHR3.
- Multiple nucleotides mutations should be done to increase the solubility of CDHR3.
- To express and purify the protein for antibody development.
- To examine the crystal structure of the protein for further studies.

References

1. Jacobs SE; Lamson DM; St George K; Walsh TJ. Human rhinoviruses. *PubMed* **(2013)**, **26**(1):135-62.

2. Bochkov YA; Watters K; Ashraf S, Griggs TF; Devries MK; Jackson DJ; Palmenberg AC; Gern JE. Cadherin-related family member 3, a childhood asthma susceptibility gene product, mediates rhinovirus C binding and replication. *PubMed* **(2015)**, **112**(17): 5485-90.