Introduction

*Pseudomonas aeruginosa* is an opportunistic pathogen that secretes a potent toxin known as *Pseudomonas* exotoxin A (PE). PE has been engineered as an anti-cancer therapeutic drug by combining it with an antibody to a tumor-associated cell surface antigen. These fusion proteins are known as recombinant immunotoxins (RITs). RITs based on PE are in clinical development for the treatment of several cancers, and one has been FDA approved for the treatment of hairy cell leukemia.

In order to kill cells, PE-based RITs must be internalized and undergo a retrograde trafficking route to reach the cytosol (Figure 2). During trafficking, RITs encounter and are cleaved by the protease furin. Furin cleaves at a specific site between PE and the antibody, separating them. This cleavage is an integral part of the intoxication pathway, but furin has been shown to be inefficient at cleaving the wild-type site on PE-based RITs.

Hypothesis

The mutations in Table I are based on amino acid usage in known furin cleavage sites. Previous studies have shown to enhance activity of RITs by improving cleavage efficiency with these mutations. Using SS1-LR/GGS, an anti-mesothelin PE-based RIT, I hypothesize that the mutations at the N-terminus of the furin cleavage site will enhance cytotoxicity, while mutations C-terminus to the furin cleavage site will not.

Results

![Figure 3. Representative Cytotoxicity Assay of SS1-LR FUR RITs with non–small cell lung carcinoma cells L55.](image)

Conclusions

We expressed, purified, and tested the cytotoxicity of furin site mutant constructs in tissue culture and found that mutations N-terminal to the cleavage site decreased cytotoxicity relative to the wild-type RIT, while mutations C-terminal to the cleavage site had minimal effect. The T-test showed a significant difference between the wildtype and the SS1-LR P’ mutations which would suggest a slight increase in SS1-LR P’ activity.

Future Directions

We plan to insert the FUR mutations into HA22-LR GGS, an anti-CD22 recombinant immunotoxin and compare its cytotoxicity to SS1-LR GGS. Previous research shows that HA22-LR GGS is more amenable to changes in the furin cleavage site.

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