**Introduction**

*Pseudomonas aeruginosa*, an opportunistic human pathogen, represents a severe threat to patients that suffer from cystic fibrosis (CF). Once chronic infection is established in CF, *P. aeruginosa* cannot be eradicated by any known treatment even when the bacteria are antibiotic sensitive when tested ex vivo. Infected patients experience periodic disease flares and persistent declines in lung function. The aminoglycoside tobramycin is one of the more effective antibiotics against *P. aeruginosa*, and treatment with tobramycin improves disease symptoms and lung function in CF patients. However, as many 10% *P. aeruginosa* clinical isolates from CF patients are tobramycin resistant, and patients with resistant organisms generally have poor outcomes.

To better understand how *P. aeruginosa* cells resist tobramycin during chronic infection, we are investigating the proteome response of *P. aeruginosa* to the presence of tobramycin.

**Methods**

- **In Vitro**
  - 1) Viability (sputum cultures)
  - 2) Data dependent acquisition (DDA) and spectral counting quantitation
  - 3) SRM and targeted quantitation
  - 4) In vivo (sputum)

**Overview**

- *P. aeruginosa* proteome response to tobramycin monitored by spectral counting and selected reaction monitoring (SRM)
- Heat shock proteins, protease, and amino acid biosynthesis related proteins in *P. aeruginosa* significantly up-regulated in the presence of tobramycin
- SRM analysis of *in vivo* *P. aeruginosa* proteins in cystic fibrosis patient samples

**Results**

**Figure 1.** Data dependent acquisition (DDA) and spectral counting quantitation were used to identify candidate proteins responsive to tobramycin treatment.

**Figure 2.** SRM assays on *P. aeruginosa* in vitro.

**Figure 3.** SRM assays on *P. aeruginosa* in vivo.

**Conclusion**

- Heat shock proteins, proteases, amino acid biosynthesis related proteins are responsive to tobramycin treatment.
- Targeted assays were used to quantify *P. aeruginosa* proteins in the sputum samples of the cystic fibrosis patients.
- Future directions: 1) Enrich *P. aeruginosa* from sputum samples of cystic fibrosis patients; 2) Develop additional SRM assays for *P. aeruginosa*.

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References:

**Xia Wu, Chunxiang Zheng, Kiara Held, Benjamin J. Staudinger, Juan D. Chavez, Chad R. Weisbrod, Jimmy K. Eng, Pradeep K. Singh, Colin Manoli, James E. Bruce* Departments of Genome Sciences, Medicine, and Microbiology, University of Washington, Seattle, WA**