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DISSERTATION

"Characterization of current and novel antibacterials against *Mycobacterium abscessus* persisters"

by

Yee-Kuen Yam

M.D./Ph.D. Program

B.S. 2015, Penn State University, University Park, PA

Thesis Advisor:
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Tuesday, March 10th, 2020
10:00 A.M.
ICPH 1st floor, TB Training Room (W140B.1)

Abstract

Nontuberculous mycobacteria pulmonary disease is a growing concern in the clinical setting with evidence of increased prevalence and lack of effective treatments. Persistence of infection despite extensive chemotherapy with antibiotics that display potent *in vitro* MIC values is a hallmark of *Mycobacterium abscessus* disease. This demonstrates a poor predictive value of the classical MIC assay for clinical outcome, even though it is the clinical standard to identify which antibiotics will be used to treat patients. Discovery of more efficacious antibiotics requires more predictive *in vitro* potency assays. As part of the *Mycobacterium* genus, *M. abscessus* is an obligate aerobe and a chemo-organo-heterotroph – it requires oxygen and organic carbon sources for growth. However, bacteria growing in patients can encounter micro-environmental conditions that are different from aerated nutrient-rich broth used to grow planktonic cultures for MIC assays. These *in vivo* conditions may include oxygen and nutrient limitation which should arrest growth. Furthermore, *M. abscessus* was shown to grow as biofilms *in vivo*. Here, we show *M. abscessus* Bamboo, a clinical isolate we use for *M. abscessus* drug discovery, can survive oxygen deprivation and nutrient starvation in non-replicating states for extended periods of time and we develop an *in vitro* model where the bacterium grows as biofilm. Using these culture models, we show that non-replicating or biofilm-growing bacteria display tolerance to clinically used anti-*M. abscessus* antibiotics, consistent with the observed persistence of infection in patients. In order to demonstrate the utility of the developed persister assays for drug discovery, we determine the effect of novel agents targeting membrane functions against these physiological forms of the bacterium and find that these compounds show anti-persister activity. Lastly, we show that *dosR*, a gene essential for hypoxia-induced dormancy in *M. tuberculosis*, is not essential for survival of *M. abscessus* in our *in vitro* persister assays. In summary, we developed *in vitro* persister assays to fill an assay gap in *M. abscessus* drug discovery and enable identification of novel lead compounds showing anti-persister activity. The observed tolerance of *in vitro* persister bacteria against clinically used antibiotics provides a possible explanation why current regimens are ineffective. The finding that the key regulator of persistence development in *M. tuberculosis*, *dosR*, is not required for persistence of *M. abscessus*, suggests that *M. abscessus* harbors a genetic dormancy program different to that of the tubercle bacillus.