Summer Student Research Program
Project Description

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PROJECT TITLE (200 Characters max):

Mechanism of Candin drug resistance in yeast pathogen *Cryptococcus neoformans*

HYPOTHESIS:

Candin drugs are the new generation antifungal drugs with fungicidal activity for several major fungal pathogens, including Candida species, Aspergillus species, and Fusarium species. However, Cryptococcus as the causative agent of deadly cryptococcal meningitis is naturally resistant to this new class antifungal drug even though the target of this drug (beta-glucan synthase) is present and functional. The mechanism of drug resistance is unknown. We hypothesize that the drug may be fail to reach the drug target because of the unique membrane structure in this pathogen, or the presence of high amount of alpha-glucan. It is also possible that a novel drug resistant mechanism is involved. This project is to understand the molecular basis of the inherited resistance of Cryptococcus to Candin drugs.

PROJECT DESCRIPTION **(Include design, methodology, data collection, techniques, data analysis to be employed and evaluation and interpretation methodology)**

Study design:
Two approaches will be employed for this project. First, we will use reverse genetics to test our hypothesis. A set of mutants that could not produce polysaccharide capsule or alpha-glucan or with altered membrane structure will be used to test the hypothesis that capsule, alpha-glucan or membrane lipids may play a role in drug resistance. The affectivity of Candin drugs (caspofungin and micafungin) in these mutant backgrounds will be tested in vitro condition using 96-well culture plates. We will also apply the forward genetics to screen a random mutagenesis library for those mutant strains failed to grow in the presence of drugs. Mutants sensitive to 8 ug/ml drugs will be selected and rescreened for their sensitivity with lower drug concentrations. A set of most sensitive mutants will be isolated and genomic DNA prepared. The mutated genes in these mutant strains will be identified by using the inverse-PCR technique. The function of identified genes will be analyzed by using bioinformatic approaches and confirmed by mutagenesis. A model will be developed on why Cryptococcus becomes naturally resistant to this drug class.

Techniques to be employed:
PCR-based diagnostic technique will be routinely used to identify gene mutations. Yeast culture growth assays will be applied to determine mutants that become sensitive to drugs. 96-well plate reader will be used to measure the growth as designed time points. Inverse-PCR technique will be used for identifying target genes of mutant strains that become sensitive to drugs. Microscopic techniques will also be used to score the in vitro phenotype of certain mutants. Data collection methods and analysis techniques: Programs Prism and Microsoft excel will be used for data analysis.

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SPONSOR'S MOST RECENT PUBLICATIONS RELEVANT TO THIS RESEARCH:

1. Masso-Silva J, Espinosa V, Liu TB, Wang Y, Xue C, Rivera A (2018) **The F-Box Protein Fbp1 Shapes the Immunogenic Potential of *Cryptococcus neoformans*.** MBio 9. PMI: 29317510
2. Liao G, Wang Y, Liu TB, Kohli G, Qian W, Shor E, Subbian S, Xue C (2018) **Role of the inositol pyrophosphate multikinase Kcs1 in *Cryptococcus* inositol metabolism.** Fungal Genet Biol. PMI: 29357302
3. Boyce KJ, Wang Y, Verma S, Shakya VPS, Xue C, Idnurm A (2017) **Mismatch Repair of DNA Replication Errors Contributes to Microevolution in the Pathogenic Fungus *Cryptococcus neoformans*.** MBio 8. PMI: 28559486
4. Huang W, Liao G, Baker GM, Wang Y, Lau R, Paderu P, Perlin DS, Xue C (2016) **Lipid flippase subunit Cdc50 mediates drug resistance and virulence in *Cryptococcus neoformans*.** MBio 7: e00478-00416. PMI: 27165800
5. Ero R, Dimitrova VT, Chen Y, W. B, Feng S, Liu T, Wang P, Xue C, Tan SM, Gao Y-G (2015) **Crystal structure of Gib2, a signal-transducing protein scaffold associated with ribosomes in *Cryptococcus neoformans*.** Scientific Reports 5: 8688. PMI:
6. Liu TB, Xue C (2014) **Fbp1-mediated ubiquitin-proteasome pathway controls *Cryptococcus neoformans* virulence by regulating fungal intracellular growth in macrophages.** Infect Immun 82: 557-568. PMI: 24478071
7. Liu TB, Subbian S, Pan W, Eugenin E, Xie J, Xue C (2014) ***Cryptococcus* inositol utilization modulates the host protective immune response during brain infection.** Cell Commun Signal 12: 51. PMI: 25201772
8. Liu TB, Wang Y, Baker GM, Fahmy H, Jiang L, Xue C (2013) **The glucose sensor-like protein Hxs1 is a high-affinity glucose transporter and required for virulence in *Cryptococcus neoformans*.** PLoS ONE 8: e64239. PMI:
9. Liu TB, Kim JC, Wang Y, Toffaletti DL, Eugenin E, Perfect JR, Kim KJ, Xue C (2013) **Brain inositol is a novel stimulator for promoting *Cryptococcus*penetration of the blood-brain barrier.** PLoS Pathog 9: e1003247. PMI: 23592982

IS THIS PROJECT SUPPORTED BY EXTRAMURAL FUNDS?
Yes ☐ or No ☒
(IF YES, PLEASE SUPPLY THE GRANTING AGENCY'S NAME)

THIS PROJECT IS: ☐Clinical ☒Laboratory ☐ Behavioral ☐ Other

THIS PROJECT IS CANCER-RELATED☐
Please explain Cancer relevance

THIS PROJECT IS HEART, LUNG & BLOOD- RELATED☐
Please explain Heart, Lung, Blood relevance

THIS PROJECT EMPLOYS RADIOISOTOPES☐

THIS PROJECT INVOLVES THE USE OF ANIMALS ☐
PENDING ☐ APPROVED ☐ IACUC PROTOCOL #

THIS PROJECT INVOLVES THE USE OF HUMAN SUBJECTS ☐

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PENDING ☐ **APPROVED** ☐ **IRB PROTOCOL # M**

THIS PROJECT IS SUITABLE FOR:

UNDERGRADUATE STUDENTS	☐	ENTERING FRESHMAN	☐
SOPHMORES	☐	ALL STUDENTS	☒

THIS PROJECT IS WORK-STUDY: **Yes** ☐ **or** **No** ☐

THIS PROJECT WILL BE POSTED DURING ACADEMIC YEAR FOR INTERESTED VOLUNTEERS?: **Yes** ☒ **or** **No** ☐

WHAT WILL THE STUDENT LEARN FROM THIS EXPERIENCE?

The student will gain hands on experience of laboratory research. The student will learn the basic concept of molecular genetics, medical mycology, as well as drug resistance. The student will learn how to design and execute experiments, as well as time management during experiments.