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 large polysaccharide capsule, or the presence of high amount of alpha-glucan compensates the cell wall defect caused by the inhibition of beta-glucan synthesis. 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 will be used to test the hypothesis that capsule or alpha-glucan 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:

SPONSOR'S MOST RECENT PUBLICATIONS RELEVANT TO THIS RESEARCH:

1. Wang, Y., Liu, T., Delmas, Park, S, Perlin, D., and **Xue, C.** (2011) Two major inositol transporters and their role in cryptococcal virulence. *Eukaryotic Cell* 10:618-628 (highlighted by the journal)
2. Liu, T., Wang, Y., Stukes, S., Chen, Q., Casadevall, A., and **Xue, C.** (2011) The F-box protein Fbp1 is essential for sexual reproduction and virulence of *Cryptococcus neoformans*. *Eukaryotic Cell* 10:791-802

3. Okagaki, L.H., Wang, Y, Ballou, E.R., O'Meara, T.R., Bahn, Y.-S., Alspaugh, J.A., **Xue, C.,** and Nielsen, K. (2011) Cryptococcal titan cell formation is regulated by G-protein signaling in response to multiple stimuli. *Eukaryotic Cell* 10: 1306-1316 (highlighted by the journal)
4. Wang, Y., Liu, T., Patel, S., Jiang, L., and **Xue, C.** (2011) The casein kinase I Cck1 regulates multiple signaling pathways and is essential for cell integrity and fungal virulence in *Cryptococcus neoformans*. *Eukaryotic Cell* 10: 1455-1464 PMID: 21926330
5. Liu, T., and **Xue, C.** (2011) The ubiquitin-proteasome system and F-box proteins in pathogenic fungi. *Mycobiology* 39: 243-248.

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 ☐
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.