Probing Molecular Mechanisms of Radioresistance: Toward Tunable Pigmentation for Passive Fungal Sensor Arrays of Radiation Exposure

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Abstract:

It is well documented that melanized fungi have a uniquely high resistance to ionizing radiation. Not only are cultures viable in extreme conditions, cell growth is enhanced upon exposure. Despite initial efforts to decouple the chemical and physical mechanisms of radioprotection from cellular growth, discrepancies in the origins of the radioresistant phenotype exist throughout the literature. Initial research comparing the radioresistance of the melanized wild type Exophiala dermatitidis and an albino mutant strain concluded that melanin was responsible for the resistant phenotype. However, subsequent efforts to quantify transcriptomic differences between the strains did not find melanin-related gene products to be tied to radioresistance. While the transcriptome is useful in comparing genetic derivatives, phenotypic differences are correlated to differences in the proteome. Still, preliminary proteomics efforts struggled to identify unique features of radioresistance but did highlight challenges associated with cell lysis.

Cells must be lysed to obtain constituent proteins, and fungi have relatively robust cell wall structure making lysis a challenge by standard procedures. Interestingly, melanin resides in the cell wall of E. dermatitidis which could have an effect on mutated strains. Optimizing a lysis procedure fit to handle the resilient cell wall of fungal strains for protein extraction independent of the strain is necessary for reproducible proteomics.

While many antifungal drugs compromise the cell wall, physical agitation is the preferred method of cell lysis so there is no effect on gene products. Several lysis methods will be tested including treatment with the antifungal drug amphotericin b, physical lysis using a bead beater, and a combination of freeze/thaw cycles with ultrasonication. The effects of the lysis method on the recovered proteome will be analyzed. Generating an array of mutants with different genetic modifications relating to melanin production can be assessed on a proteomic level.