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EDITORIAL

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Targeting protein localization for anti-infective therapy

J. Andrew Alspaugh

Department of Medicine, and Department of Molecular Genetics & Microbiology, Duke University School of Medicine, Durham, NC, USA

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Proteins rarely function in isolation. Instead, they tend to operate in pathways and complexes, interacting with other factors in the cell. One of the main ways in which these interactions are maintained is the precise, regulated localization of proteins to specific subcellular domains.

Some proteins have intrinsic molecular features that favor their localization to particular cellular sites. These features include hydrophobic transmembrane domains, nuclear importation signals, and signal sequences that promote secretion. Other proteins require post-translational modification (PTM) for appropriate subcellular localization. One such family of related PTMs is the addition of hydrophobic lipid groups that target proteins to membranous structures.[1] These post-translational lipidation reactions include palmitoylation, farnesylation, and geranylgeranylation. Although many proteins undergo this type of post-translational lipidation, Ras-like proteins have been extensively documented to require lipidation and membrane-association for function.[2,3]

Given the importance of Ras proteins in tumor cell biology, extensive efforts have explored human farnesyltransferase (FTase) proteins as targets for anticancer therapies.[4] Although farnesyltransferase inhibitors (FTIs) demonstrate anti-proliferative effects in many Ras-mediated malignancies, their efficacy in in clinical trials has been somewhat limited, perhaps due to compensatory protein lipidation by other enzymes, such as geranylgeranyltransferases.[5]

In addition to applications in cancer therapy, FTIs have also been explored as novel antimicrobial agents. Compounds that demonstrate FTI activity in human cells can also inhibit fungal growth in vitro.[6,7] This type of pharmacological farnesyltransferase inhibition has also been extended to genetic studies. Mutation of the consensus CAAX sequence for protein lipidation results in dysfunctional fungal Ras-like proteins .[8,9] As a result, these lipidation-defective fungal mutants tend to display reduced stress tolerance, often manifested by a reduced ability to grow at 37°C[7-11]

In a recent manuscript, Norton and colleagues explore the role of protein farnesylation in the growth, development, and virulence of the human fungal pathogen *Aspergillus fumigatus*.[12] This ubiquitous filamentous fungus is a common inhabitant of many temperate environments. Additionally, *A. fumigatus* is an important opportunistic pathogen, causing invasive disease in highly immunocompromised patients. Normally inhibited by innate immune cells, spore germination by *A. fumigatus* results in the production of highly polarized hyphal cells. These filamentous structures are able to penetrate and damage host tissue. Additionally, the infection can spread in the bloodstream to distant sites. Both the local tissue destruction and metastatic dissemination result in serious, and potentially life-threatening, infections in vulnerable, immunosuppressed patient populations.

The investigators of this recent study described previously the importance of Ras proteins in proper *A. fumigatus* hyphal formation and virulence.[13-16] Ras-like proteins are small, monomeric guanine nucleotide-binding proteins that serve as molecular switches to regulate many central cellular processes. For example, the *A. fumigatus* RasA protein is required for radial hyphal growth in vitro, as well as for growth and dissemination in vivo using animal models of aspergillosis.[14] This protein is thought to direct the polarization of particular proteins to the growing hyphal tip. However, RasA, like all Ras-family proteins, must itself be localized to membranes to properly function. The initial membrane association event is directed by protein farnesylation, and mutation of the consensus CAAX sequence for

CONTACT J. Andrew Alspaugh andrew.alspaugh@duke.edu Duke University School of Medicine, DUMC 102359, 303 Sands Building, Research Drive, Durham, NC 27710, USA.

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farnesylation of RasA results in an improperly localized and dysfunctional protein.[9]

In this new manuscript, the investigators disrupt the *ramA* gene encoding the β-subunit of the dimeric protein FTase.[12] As predicted for this lipidation-impaired strain, the *ramAΔ* mutant strain fails to properly localize Ras proteins to membranes. Accordingly, the *ramAΔ* mutant phenotypically mimics the *rasAΔ* mutant strain, with both strains displaying reduced radial growth rates, altered hyphal polarity/nuclear distribution, and attenuated virulence. However, these genetic studies also suggest that the Ram1 protein has targets in addition to classical Ras proteins. For example, the *ramAΔ* farnesyltransferase mutant has an irregular pattern of nuclear distribution into conidia, a feature not shared with *ras* mutants. Additionally, the *ramAΔ* mutant displays a unique altered resistance to triazole antifungal drugs.

These recent investigations support a growing body of literature that demonstrates how conserved cellular processes, such as Ras protein localization, might be targeted for therapeutic effect. Principles of FTase substrate specificity and cross-lipidation have been documented in mammalian systems, and these studies are directly applicable to analogous cellular processes in microorganisms. Moreover, fungal-specific features in farnesyl/geranylgeranyltransferase proteins have been identified using X-ray crystallography.[6,17] Therefore, it is very likely that compounds can be developed to specifically target mammalian or microbial enzymes. However, many challenges remain regarding developing microbe-specific FTIs, include ensuring that compounds with potent activity against FTases are pharmacologically optimized and can stably enter the target cell. The recent manuscript by Norton, *et al.* provides further impetus to explore this exciting and promising path for new anti-infective therapies.

Disclosure of potential conflicts of interest

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