Homework Questions due at the start of class 7.31

"In vitro and in vivo antimalarial activity of peptidomimetic protein farnesyltransferase inhibitors with improved membrane permeability" by D. Carrico et al. (PubMed ID 15556768)

Questions by Courtnee Clough, Avrey Novak, Emily Boevers

Read the paper above and cite additional sources as needed. Generally, this paper aims to assist in understanding of the 'hit-to-lead' process in drug development. Specifically, this paper demonstrates the significant impact chemical structure has on effectiveness and the difficulties of repeating target based results in vivo.

1. What is prenylation and its biological significance?

Prenylation is the attachment of 5-carbon prenyl groups (often combined into C_{15} or C_{20} groups) to proteins or other molecules. Prenylation appears important in anchoring proteins to the cell membrane and in mediating certain protein-protein interactions (in which prenyl groups addto the strength of the binding).

2. Refer to the compounds in Figure 1. What is the difference between PFT inhibitors FTI-2148 and FTI-2153 in terms of structure? What do they hypothesize accounts for their difference in antimalarial activity?

The R group is –H for FTI-2148 and $–CH_3$ for FTI-2153, making the corresponding structures a carboxylic acid and a methyl ester, respectively. The authors hypothesized that the greater hydrophobicity of the ester allowed it to cross membranes and thus kill the parasite more effectively.

3. Why was the drug effectiveness studied in mammalian cells?

This is an open-ended question whose answer is not given explicitly in the paper, so any reasonable speculation is acceptable. Coauthor Fred Buckner said that the Yale lab contributing to this work was interested in cancer drug development, so studying human cells made sense in that context.

4. Generally, researchers clone a gene of interest in order to collect large amounts of protein. In other experiments, expression of 1000 Plasmodium genes gave only ~6% success rate for soluble protein. (For more information, consult "Heterologous expression of proteins from Plasmodium falciparum: results from 1000 genes." PubMed ID 16644028) How did the authors avoid this problem?

The problems of recombinant Plasmodium protein expression were avoided by purification of the naturally occurring protein from Plasmodium cells using ammonium sulfate fractionation or Mono Q chromatography, as described in reference #17.

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5. Complete the BQMOC Process for Figure 2.

B: Increasing the hydrophobicity of farnesyltransferase inhibitors may improve their ability to penetrate cells and inhibit farnesylation. Q: Will ester forms of the compounds inhibit farnesylation more effectively than the free acid? M: Mouse fibroblast cells were treated with the test compounds, then lysed. Proteins were separated via SDS-PAGE, then transferred to nitrocellulose filters, and Ras and Rap1A were detected with antibodies. O: Ras and Rap1A were mostly "processed" (containing prenyl groups) when exposed to low concentrations of the free acid (1-10 nM), whereas esters at these concentrations led to accumulation of the "unprocessed" proteins (not containing prenyl groups). C: The esters did indeed inhibit farnesylation at lower concentrations than the free acid.

6. There have been previous papers with successful lab results but unsuccessful cellular level therapies. What challenges might be encountered with this therapy? Consult this paper, outside sources or the paper, "Efficacy, pharmacokinetics, and metabolism of tetrahydroquinoline inhibitors of Plasmodium falciparum protein farnesyltransferase." (PubMed ID 17606674)

PubMed ID 17606674 demonstrates that even when a compound is able to enter cells and kill parasites, its effectiveness might be limited if it is rapidly metabolized and/or eliminated by the host. Other potential challenges include toxicity to host cells.