Role of HAD2 in Metabolic Homeostasis and Drug Resistance in Malaria Parasite *Plasmodium falciparum*

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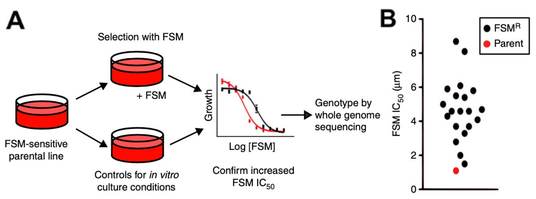
Background and Hypothesis

Malaria caused by the parasite *Plasmodium falciparum* is responsible for approximately half a million deaths annually. The development of widespread resistance to antimalarials prompts an urgent need for novel therapeutics and diagnostics, which require a deeper understanding of malaria parasite biology.

P. falciparum uses the methylerythritol (MEP) pathway to synthesize isoprenoids, a group of biomolecules that perform essential cellular functions. Because this pathway is essential and distinct from the human pathway of isoprenoid biosynthesis, it is an ideal target for antimalarials.[3] Through a selection screen, the John Lab generated parasites resistant to the MEP pathway inhibitor fosmidomycin (FSM) and sequenced them to find a single nucleotide polymorphism (SNP) in the gene encoding for the protein HAD2.[4] This SNP (R157X) resulted in a truncated protein predicted to be nonfunctional. This strain (*had2*^{R157X}) was both FSM-resistant and growth-attenuated (relative to the wild-type strain).

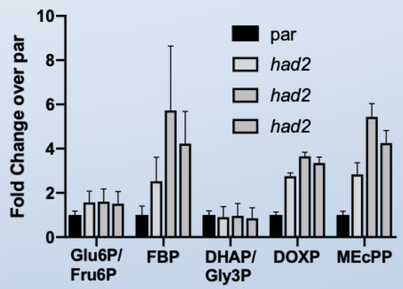
Therefore, we *hypothesize* that HAD2 is required for glycolytic and isoprenoid biosynthesis homeostasis in *P. falciparum*.

Generating FSM-resistant Parasites



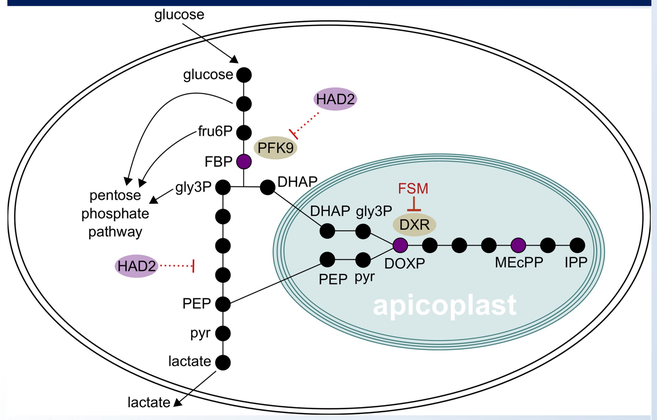
Source: Guggisberg et al. 2014

had2^{R157X} Overcomes Competitive Inhibition by FSM



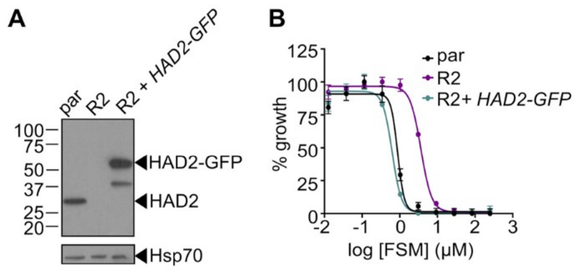
Metabolic profiling of *had2* strains showed increased levels of the MEP pathway intermediates DOXP and MEcPP and the glycolytic pathway intermediate FBP.
Source: Guggisberg et al. 2018

HAD2-Mediated Metabolic Regulation



Source: Guggisberg et al. 2018

HAD2-GFP Complements *had2*^{R157X}

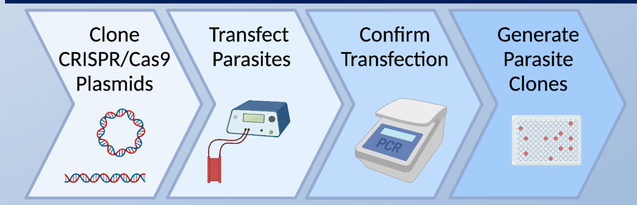


Expression of HAD2-GFP in *had2*^{R157X} (R2 + HAD2-GFP) restored FSM sensitivity.
Source: Guggisberg et al. 2018

Objectives

1. Develop a strain of *P. falciparum* with an inducible knockdown (KD) of HAD2.
2. Characterize how loss of HAD2 impacts parasite growth, metabolism, and drug resistance.

Generating Knockdown Parasites



Results

Figure 1. **The DOZI-TetR aptamer system controls protein expression.** Unless aTc is bound to TetR, DOZI-TetR interacts with hairpin aptamers in the mRNA to inhibit translation.

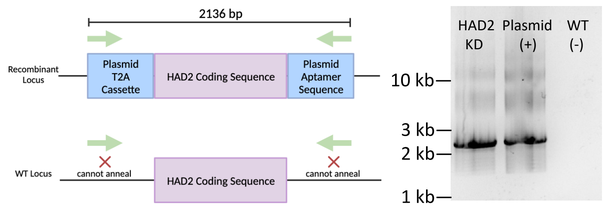


Figure 2. **Successful transfection of HAD2 KD parasites.** PCR tests confirm genomic integration of the knockdown plasmid in the HAD2 KD parasite strain.

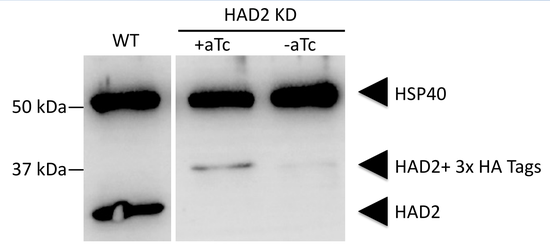


Figure 3. **Knockdown of HAD2 in HAD2 KD parasites.** Western blotting demonstrates the HAD2 KD expresses intermediate levels of HAD2 protein when on aTc, and nearly no HAD2 protein when off aTc. The blot was probed with anti-HAD2 (expected masses: HAD2, 33kD; HAD2 + 3x HA Tags, 36.5 kD) and HSP40 as a loading control.

Conclusions

1. Saturating concentrations of aTc do not produce wild-type levels of HAD2 in HAD2 KD parasites.
2. Intermediate levels of HAD2 protein correspond to intermediate growth and resistance phenotypes.
3. HAD2 may be a "Goldilocks" protein: FSM resistance does not increase when protein levels drop below an unspecified threshold.

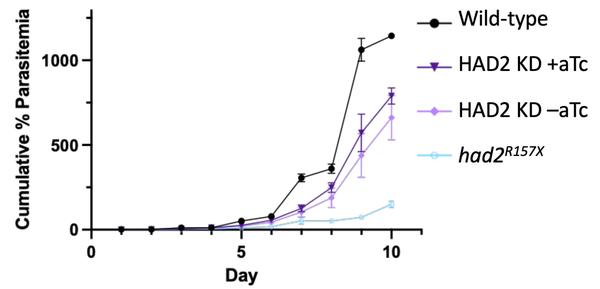


Figure 4. **HAD2 KD parasites exhibited reduced growth rates relative to the wild-type strain**. Adding aTc did not fully alleviate this defect. Notably, the growth defect in the HAD2 knockdown strain was not as pronounced as the *had2*^{R157X} strain. Data represents mean +/- SEM of technical replicates.

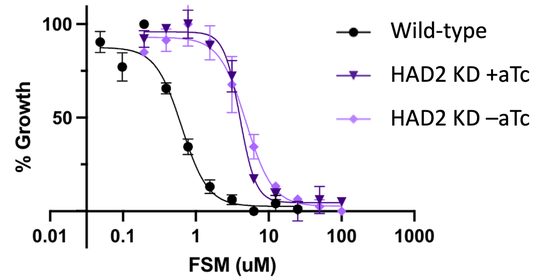


Figure 5. **HAD2 parasites are FSM-resistant.** HAD2 KD parasites exhibited increased FSM resistance (6.4-7.6 times higher than wild-type) as quantified by dose-inhibition assays. Data is normalized such that 100% growth is defined by that of untreated cells and 0% growth is defined as the smallest value in each data set. Data represents mean +/- SEM of technical replicates.

Next Steps

1. Whole genome sequencing
2. Metabolite profiling

Acknowledgements & References

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Guggisberg AM, Frasse PM, Jezewski AJ, Kafai NM, Gandhi AY, Erlinger SJ, Odom John AR. 2018. Suppression of drug resistance reveals a genetic mechanism of metabolic plasticity in malaria parasites. mBio 9:e01193-18.

Guggisberg AM, Park J, Edwards RL, Kelly ML, Hodge DM, Tolia NH, Odom John AR. 2014. A sugar phosphatase regulates the methylerythritol phosphate (MEP) pathway in malaria parasites. Ncomms 5:4467. DOI: 10.1038/ncomms5467