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ISID Research Grant Report

Functional genomics analyses of the differential susceptibility of early and late P. falciparum *gametocytes to antimalarial compounds in order to aid drug discovery efforts*

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Introduction

Malaria contributes significantly to the global disease burden, resulting in ~438 000 deaths in 2015 (1). This disease is caused by the *Plasmodium* parasite which is transmitted by a mosquito vector and infects the red blood cells of its human host before rupturing them in a continuous 48 hour cycle (2). In combatting malaria, historical efforts were geared towards reducing mortality by targeting the pathogenic, asexual stages of the parasite (3,4). With the advent of the malaria eradication agenda in 2007, research has shifted towards investigating therapies for preventing malaria transmission (5). Transmission of the parasite from one person to another results from a subset of asexual parasites differentiating into long-lived gametocyte stages that once mature, circulate in the blood to be taken up by a feeding mosquito (6,7). These parasites are sufficiently diverged from their asexual counterparts that they show limited or no susceptibility to available chemotherapies, resulting in few, not necessarily potent, interventions against transmission (8–11). A further complication of targeting gametocyte stages are differences in metabolism during early and late stages of development that result in different levels of susceptibility to chemical compounds (8,12,13). This study aimed to characterise the molecular mechanisms behind the differential activity of these compounds on a global transcriptomic level. The data provide insight into the largely uncharacterised biological response of the metabolically quiescent (13,14), late stage gametocyte to chemo-intervention as well as the biological activities being targeted by each of these compounds.

Materials and Methods

Plasmodium falciparum gametocytes were cultured in human red blood cells using an established culture system (15). Dose response and rate of activity for each of the compounds were determined on both early and late stage luciferase-expressing gametocytes, using a luminescence based assay (16). Data concerning the dosage and rate of activity for each of these compounds were used to determine the concentration and duration of treatment for transcriptomic sampling. Following treatment at 10X the effective inhibitory concentration (which decreases the parasite population's viability by 50%), samples were taken at 24 hours and 48 hours post treatment. Microarray analysis was conducted in partnership with the Manuel Llinás laboratory at the Pennsylvania State University, USA. Each treatment condition was compared to an untreated population that was cultured in parallel and key differences in the transcriptomic profiles were evaluated by both targeted searching and network analysis based approaches..

Results and Discussion

Killing rate and stage-specific activities of gametocytocidal compounds

Dose response evaluation revealed that compound 1 inhibited early gametocyte viability by 50% at 600 ± 88 nM and late-stage gametocyte viability at 180 ± 8 nM, indicating preferential late-stage gametocyte activity. By contrast, compound 2 inhibited early gametocyte viability

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by 50% at 4.5 ± 3.6 nM and late-stage gametocyte viability at 28.7 ± 0.2 nM, indicating increased susceptibility during the early stages of gametocytogenesis. Both compounds exhibited a slow rate of activity, with compound 1 still showing 60-90% viability by 24 hours post treatment and a reduction to 0-20% viability after 72 hours post treatment. Following treatment with compound 2, no measurable reduction in viability was seen after 24 hours post treatment, while viability was reduced to between 40-60% 72 hours post treatment. In order to assess changes in the transcriptome before and during visible reduction in viability, 24 and 48 hours post treatment were chosen as time points for microarray analysis.

Global transcriptional response of *P. falciparum* gametocytes to drug treatment

Global correlations between the untreated and treated profiles of *P. falciparum* gametocytes were determined by Pearson correlation. This revealed a lower correlation between untreated late-stage gametocytes and late-stage gametocytes treated with compound 1 (r2= 0.57) than was seen for early-stage gametocytes (r2=0.69). By contrast, compound 2 showed a closer correlation with untreated parasites both during early (r2= 0.75) and late (r2= 0.71) stages of gametocyte development. This would imply that both early and late stages of gametocyte development respond at a transcriptional level following drug treatment, although it still needs to be investigated whether this response is comparable to what is observed in asexual parasites (17,18).

Biological mechanisms regulating perturbation responses

Chemotype-specific responses to each compound were investigated by determining which subset of genes were significantly (t-test, P<0.05) affected regardless of the stage being targeted. Genes that were uniquely affected following treatment with each compound were subjected to Gene ontology enrichment analysis to probe into enriched biological processes affected by each compound (Figure 1).

Compound 1 Compound 2

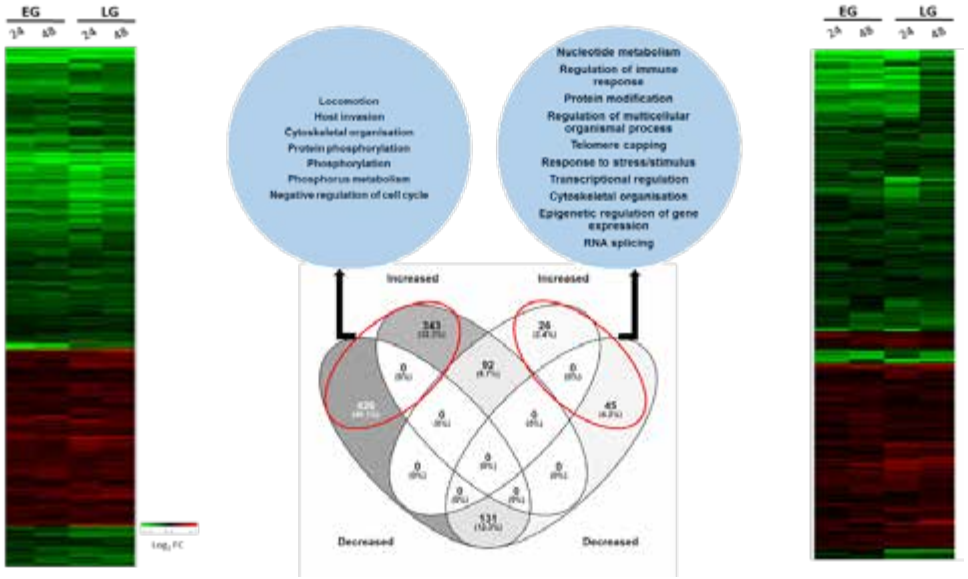

Figure 1: Transcriptional response of gametocytes under drug treatment. Heat maps show log2 fold change in expression of significantly affected genes (P<0.05) following drug perturbation of early-

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stage gametocytes (EG) or late-stage gametocytes (LG). The Venn diagram highlights unique and shared significantly affected genes between compounds with enriched biological processes (Gene ontology enrichment) following treatment with each compound indicated in the blue circles.

Further characterisation of the drug response involved constructing gene association networks using STRING (19) as well as targeted searching by investigating clusters of known signalling molecules. For compound 1, a clear pattern already emerged from the gene association networks indicating the compound greatly decreases expression of a number of protein kinases that were expressed preferentially during the late stages of gametocyte development. Compound 2 did not elicit a response that could be linked to stage-specific expression during development, linking to the more comparative correlation seen on the global transcriptomic profile. To determine whether effects on specific subsets of cellular signalling molecules (kinases, phosphatases, epigenetic factors, transcription factors) could have resulted in the different susceptibility of the gametocyte stages, the change in expression for each of these clusters were investigated. While compound 1 once again primarily affected kinases and phosphatases that were primarily expressed during the later stages of development, a different pattern of expression was seen for compound 2. Treatment with compound 2 seemed to primarily affect epigenetic factors, including histones and histone modifying enzymes, in line with the gene ontology enrichment predictions.

Conclusions

This work describes the *P. falciparum* gametocyte's response to specific drug perturbation. We provide, for the first time, an in-depth characterisation of the stage-specific responses that lead to differential drug susceptibilities in this life cycle stage of the parasite. Furthermore, the detailed description of molecules affected by the activity of these potent gametocytocidal compounds will aid in their mode-of-action deconvolution to enable progression of established drug discovery pipelines. This work is currently being prepared as part of a manuscript for submission to a peer-reviewed journal.

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