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Research paper

In silico multiple-targets identification for heme detoxification in the human malaria parasite *Plasmodium falciparum*

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ABSTRACT

Detoxification of hemoglobin byproducts or free heme is an essential step and considered potential targets for anti-malaria drug development. However, most of anti-malaria drugs are no longer effective due to the emergence and spread of the drug resistant malaria parasites. Therefore, it is an urgent need to identify potential new targets and even for target combinations for effective malaria drug design.

In this work, we reconstructed the metabolic networks of *Plasmodium falciparum* and human red blood cells for the simulation of steady mass and flux flows of the parasite's metabolites under the blood environment by flux balance analysis (FBA). The integrated model, namely iPF-RBC-713, was then adjusted into two stage-specific metabolic models, which first was for the pathological stage metabolic model of the parasite when invaded the red blood cell without any treatment and second was for the treatment stage of the parasite when a drug acted by inhibiting the hemozoin formation and caused high production rate of heme toxicity. The process of identifying target combinations consisted of two main steps. Firstly, the optimal fluxes of reactions in both the pathological and treatment stages were computed and compared to determine the change of fluxes. Corresponding enzymes of the reactions with zero fluxes in the treatment stage but non-zero fluxes in the pathological stage were predicted as a preliminary list of potential targets in inhibiting heme detoxification. Secondly, the combinations of all possible targets listed in the first step were examined to search for the best promising target combinations resulting in more effective inhibition of the detoxification to kill the malaria parasites. Finally, twenty-three enzymes were identified as a preliminary list of candidate targets which mostly were in pyruvate metabolism and citrate cycle. The optimal set of multiple targets for blocking the detoxification was a set of heme ligase, adenosine transporter, myo-inositol 1-phosphate synthase, ferrodoxim reductase-like protein and guanine transporter. In conclusion, the method has shown an effective and efficient way to identify target combinations which are obviously useful in the development of novel antimalarial drug combinations.

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1. Introduction

Malaria is one of the most devastating infectious diseases, affecting hundreds of millions of people in tropical and sub-tropical countries worldwide (WHO, 2011). There are five species of the malaria parasites infecting humans, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi*. Of the five species, *P. falciparum* is the most deadly parasite and accounts for most of severe malaria diseases and deaths. When the malaria parasite infects its host, the parasites will first develop in the liver, without causing any treatment symptoms. Thereafter, the parasites are released from liver into the blood circulation and enter the human red blood cell. Following the red blood cell invasion, the parasite grows and uptakes hemoglobin and others nutrients. The degradation of hemoglobin leads to the production of toxic byproducts or hemes. During the pathological condition, free heme is then converted into hemozoin, the malaria pigment. Once the parasite completes its replication cycle, they cause red blood cell rupture and release pigments into the blood circulation. The continuation of the development stages of malaria parasites in red cells can contribute to the malaria symptoms. Most of anti-malaria drugs are highly effective in killing the blood stage malaria parasites. This include the clinical antimalarial drugs, such as chloroquine and mefloquine (malERA, 2011; Mauritz et al., 2009; WHO, 2011), which are thought to kill malaria parasites by inhibiting hemozoin production. However, these anti-malaria drugs are no longer effective for malaria treatments due to the emergence and spread of the drug resistant malaria parasites. Therefore, it is an urgent need to identify potential new targets for an effective malaria treatment. The systematic search and analysis of the heme detoxification pathway and related metabolic pathways may allow identification of novel targets for drug development.

Currently, the genomes of the human malaria parasites *P. falciparum* and *P. vivax* have been published. The genome sequence revealed more

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than 5000 genes, more than 50% of which encode proteins of known functions (hypothetical proteins). Using the transcriptomic and proteomic tools, approximately 3000 genes (60% of genes in the genome) are shown to be expressed during the pathogenic blood stages. Using yeast two-hybrid assays, almost 3000 protein–protein interaction were identified. Together, these large-scale genomic studies provide useful resources for identification of novel targets for anti-malaria drug development (Alam et al., 2009; Fatumo et al., 2009; Huthmacher et al., 2010; Lewis et al., 2010; Ludin et al., 2012; Olszewski et al., 2009; Rout et al., 2014). Biochemical analysis and genetic manipulation (e.g. gene knockout/knockdown) are considered the conventional approaches for determining functions and essentiality of the parasite's proteins, although these approaches can be costly, time-consuming and labor-intensive. Alternatively, in silico approaches have been recently developed for prioritizing potential targets. In one study, the genome sequence of *P. falciparum* was used to construct a pathway/genome database called PlasmoCyc (Caspi et al., 2015). Then, a computational algorithm was developed to search a "chokepoint" reaction and identified >200 "chokepoint" enzymes that may serve as potential metabolic drug targets (Yeh et al., 2004; Fatumo et al., 2009). Another method based on network topology has been developed to detect potential drug targets both in solely *Plasmodium* and in *Plasmodium* with the human host enzyme (Fatumo et al., 2011) The research showed that the model with only known coding genes is an ideal network for identifying drug targets. In the meanwhile, a linear programming technique, Flux Balance Analysis (FBA), has been developed for simulating metabolism in genomic scale reconstructions of many metabolic networks (Becker and Palsson, 2008; Huthmacher et al., 2010; Orth et al., 2010; Plata et al., 2010). Therefore, presently several enzymes, transporters and proteins of the parasites have been identified conventionally and computationally as potential drug targets. Most well-studied pathways of the parasites are heme detoxification, nucleic acid metabolism, oxidative stress and fatty acid biosynthesis (Alam et al., 2009; Baragana et al., 2015; Ludin et al., 2012). Specifically, hemozoin in heme detoxification pathway, and plasmepsin, falcipain and aminopeptidase in hemoglobin degradation pathway are potential targets in food vacuole where many essential metabolic processes of the parasite survival are present (Alam et al., 2009; Baragana et al., 2015; Law et al., 2013). Many targets of interest are also found in nucleic acid regulation pathway such as mitogen related kinase whose inhibitor was proposed and examined recently (Geyer et al., 2009). In protein synthesis, glutamyl-tRNA(Gln) amidotransferase were a chokepoint identified as a potential target catalyzing glutamyl-tRNA Gln into glutminyl-tRNA (Gln) (Plaimas et al., 2013). Recently, an inhibitor of glutathione synthetase, playing an important role in defensive, has been designed and test in the laboratory (Kumar et al., 2015). A bifunctional enzyme like S-adenosylmethionine decarboxylase/ornithine decarboxylase for polyamine synthesis is also known as an important target for the parasite cell growth in the absence of exogenous polyamines (Dholakia et al., 2015). Thioredoxin reductase which is responsible for defending against oxidative damage has been proposed as a new target recently. Finally, many lipid synthesis enzymes represent potential drug targets (Gulati et al., 2015). A broad screening assay in the global lipid landscape has been performed recently in (Gulati et al., 2015). However, due to the development of drug resistant parasite and lack of analysis model for resistant stage or mechanism obviously, the analysis based on metabolic network is still important to study and identify novel either single or multiple potential targets against the resistant condition.

Based on metabolic network analysis, FBA has been widely used to predict essential genes of the human malaria parasite *P. falciparum* and other species (Dholakia et al., 2015; Huthmacher et al., 2010; Orth et al., 2010; Plata et al., 2010). In addition, there are several modifications on its linear programming technique to make the static metabolic model more realistic and useable. For example, the gene expression profiles of the malaria parasite can be integrated into the metabolic models (Dholakia et al., 2015; Huthmacher et al., 2010; Plata et al., 2010). This allowed the reconstruction of stage-specific metabolic network for different stages of the parasites and presented an opportunity to find drug targets of certain stages. More recently, host-parasite interactions can also be studied using FBA (Huthmacher et al., 2010). The metabolic processes of *Plasmodium* in an infected red blood cell has been analyzed and shown the interactions between the host's and the parasite's metabolites exchanging in the metabolic system (Mauritz et al., 2009; Olszewski et al., 2009). Recently, the development of two-stage flux balance analysis has been proposed to identify drug targets by comparing the differences of flux flows between pathological stage and medication stage (Li et al., 2011). With the development of drug resistant parasite, during the blood stage the human enzymes may help the parasite to resist the old and develop themselves to stay alive (malERA, 2011). Therefore, we here reconstructed global metabolic network of blood stages *P. falciparum* under the pathological stage condition and treatment stage condition and analyzed the mass flows for identifying the effective combination of drug targets. Especially, by mimicking the detoxification process of the parasite we employed a large-scale metabolic model of *P. falciparum*, including the hemozoin formation, and proposed the promising combinations of drug targets whose inhibition will give the same effect but more effective in the malaria treatment by targeting the heme detoxification.

2. Methods

2.1. The workflow diagram

The workflow diagram of our method is depicted in Fig. 1. Firstly, the genome-wide metabolic model of *P. falciparum* iTH366 (Plata et al.,

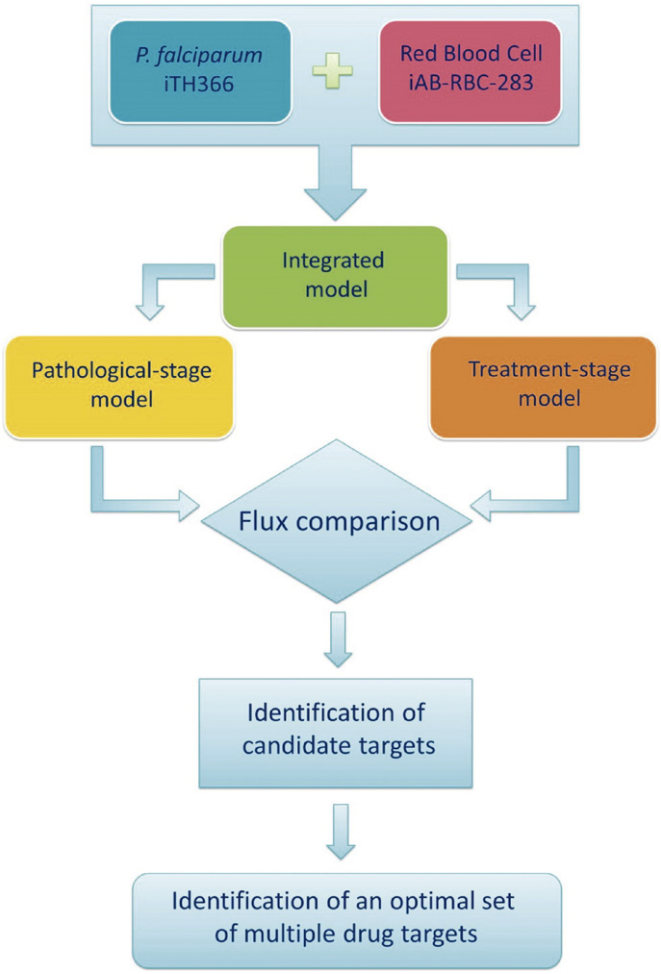

Fig. 1. The workflow diagram.

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