Microbes and Infectious Diseases 2025; 6(4): 6819-6827

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Review article

Current advances in the application of OMICS technologies against malaria

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ARTICLE INFO

Article history:
Received 26 February 2024
Received in revised form24March 2024
Accepted 26 March 2024

Keywords:
Proteomics
Plasmodium
Genomics
Transcriptomics
Metabolomics

ABSTRACT

Background: Malaria is an infectious disease caused by intracellular parasites of the genus *Plasmodium*. Despite being preventable and treatable, malaria remains one of the major health problems in sub-Saharan Africa. *Plasmodium falciparum* is the most prevalent malaria parasite in the African continent. It is responsible for most malaria related deaths globally. *Plasmodium vivax* is the dominant malaria parasite in most countries outside of sub-Saharan Africa. A complete understanding of the biology of malaria parasites is challenging in view of their need to switch between the vertebrate and insect hosts. Although knowledge of genomic sequences for the malaria parasites, *Plasmodium falciparum* and *Plasmodium vivax*, have helped to advance our understanding of malaria biology, simply knowing this sequence information has not yielded many new interventions to reduce the burden of malaria. Application of OMICS technologies in the fight against malaria will significantly advance our understanding of parasite biology and knowledge about parasite genetic diversity which can be used to aid discovery of vulnerable targets for intervention strategies, such as drugs, vaccines or insecticides, and to identify biomarkers for diagnosis, surveillance and monitoring of malaria infections in humans or mosquitoes. In this review, current information of how the knowledge of omics technologies is applied in the fight against malaria is provided.

Introduction

Malaria is caused by *Plasmodium* genus, with *P. falciparum*(*P.falciparum*)and *P. vivax* causing most cases. Mosquitoes transmit the parasites as sporozoites into the host's blood-stream, before invading liver cells and undergoing a rapid growth and division phase as schizonts [1]. The liver cells eventually rupture, releasing these parasites into the blood-stream as non-motile merozoites, to begin the asexual stage of infection. A subset of asexual blood-stage parasites subsequently develops into male and female gametocytes, which can be picked up by mosquitoes and transmitted to other hosts [2]. When biting an infected human host, the mosquito takes in gametocytes as part of the blood meal. Within the midgut, male and female gametocytes fuse, forming oocysts which then bud to become sporozoites [3]. The highly mobile sporozoites migrate to the salivary gland, where they are poised to invade a new human host during a subsequent blood meal [4]. Once in a human host, sporozoites migrate to the liver and invade hepatocytes, where they replicate and release thousands of merozoites into the blood [4]. Merozoites invade erythrocytes where they differentiate into trophozoites and begin a cycle of replication, explosive release, and reinvasion, leading to the periodic nature of malaria symptoms.

DOI:10.21608/MID.2024.266650.1826
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Periodically, some merozoites differentiate into male and female gametocytes for transmission through the mosquito host, thereby completing the complicated three-way life cycle [5].

Globally in 2022, there were an estimated 249 million malaria cases and 608 000 malaria deaths in 85 countries [6]. A range of complementary intervention strategies to control the parasite is currently in use, including mosquito habitat disruption, domestic insecticide application, insecticide-treated bed nets, anti-malarial drugs, etc. [7]. Generally, these approaches are reasonably successful and cost-effective; for example, long-lasting insecticide-treated bed nets can remain effective without re-treatment following six years of use [8]. However, the resurgence in the disease is being seen in many parts of the world, due in part to increasing resistance to antimalarials and insecticides.

Conventional technologies are poorly suited to identifying drug targets through comprehensive analyses of an organism's biology and drug mechanisms [9]. A promising alternative involves applying the suite of "omics" technologies, e.g., genomics, transcriptomics, proteomics, metabolomics, and glycomics, to develop a multi-faceted profile of the parasite's inner workings to locate its most vulnerable points. The "omics revolution" has fostered an ambitious, data-intensive approach to biology that emphasizes large-scale, aggregate studies using automation, high-throughput techniques, and sophisticated data mining methodologies.

Although knowledge of genomic sequences for the malaria parasites, *Plasmodiumfalciparum* and *P. vivax* , has helped advance our understanding of malaria biology, simply knowing this sequence information has not yielded new interventions to reduce the burden of malaria. Here we review and provide information on how the knowledge of omics technologies is applied in the fight against malaria.

Genomics

Genomics employs DNA sequencing, assembly, and annotation to document the genome of an organism in its entirety [10]. The successful sequencing of the *Plasmodium* genome resulted in the identification of a range of genes related to essential processes for the parasite's survival, virulence, and mutagenesis. These genes and their products are therefore potential new antimicrobial targets [11]. This knowledge is of utmost importance to the development of new screening platforms for the identification and selection of compounds targeting specific essential genes based on protein interactions and mutant libraries [12]. Sequencing methods can also be applied in the discovery of potential targets through the selection of mutants resistant to the new compound, followed by genetic comparison [13]. In this case, the wild-type strain is cultivated in the presence of the antimicrobial compound, and mutants can be generated by natural selection. Another system developed from genomic technologies is based on the screening of a genome-wide mutant library [14]. These mutants can be generated through mutagenesis mediated by mobile genetic elements such as transposons [15]. Next, mutants with altered sensitivity to the compound are identified, elucidating the pathways involved in its action (through genomic studies). This approach was adopted to elucidate the molecular mechanism of alfalfa snakin-1 (MsSN1), an antimicrobial peptide produced by *Medicag osativa* , against *Pseudomonas fluorescens* Pf-5.

Genome studies have discovered that single-point mutations in many genes of *Plasmodium* are associated with drug resistance. For instance; mutations in the kelch13 gene were associated with slow-elimination parasite clearance (RCA) in malaria patients treated with artemisinin-based combination therapy (ACT) [16]. This can become a very serious problem in the fight against malaria since artemisinin and its derivatives are currently the most powerful drugs used against the disease [17]. Similarly, mutations in other genes of *Plasmodium falciparum* such as; the *pfcrt*, *pfmdr1*, *pfdhfr*, *pfdhps* have been associated with drug resistance.

It has been found through comparative genomics with other *Plasmodium* sp, that genes in the chromosomal cores of *Plasmodium* species are relatively conserved, unlike genes in the subtelomeric regions which are highly species-specific. This may be because subtelomeric regions are generally unstable and are associated with a high rate of recombination as well as nucleotide loss and disruption. Genes in this region are often involved in host–parasite interactions (e.g. invasion) and may therefore trigger host immune responses, perhaps leading to diversifying selection as a result of immune pressure [5].

The *var* gene family encoding the *P.falciparum* erythrocyte membrane protein 1 (PfEMP1) surface protein is one of the most well-studied subtelomeric gene families, this is due to its association with parasite virulence and immune evasion [18]. Located mainly in the variable region on the chromosomal peripheries, var genes undergo recombination at a rate eight times higher than average. Furthermore, genes of this family appear to undergo inter-chromosomal recombination as well, leading to a diverse repertoire of antigenic genes [19]. Genomics can help reveal the extent of antigenic variation in this family, thereby aiding the goal of discovering broadly representative patterns that can be targeted in new interventions. Sequencing of the *P. falciparum* genome revealed that the var genes could be organized into five subgroups depending on the upstream flanking sequence (UpsA to UpsE). This information will be pivotal in understanding how var gene transcription is regulated [20]. Classification of the var genes has also enabled researchers to single out unique var genes that fall outside the main groups. For example, during pregnancy-associated malaria, var2CSA is believed to be the principal ligand that binds to chondroitin sulfate A in the placental endothelial lining [21]. This type of knowledge could prove to be useful in identifying stage-specific malaria vaccine targets.

Post *Plasmodium falciparum* and *Plasmodium vivax* genome sequencing expectations

The publication of the *P. falciparum* genome sequence in 2002 [22] followed by the *P. vivax* genome sequence in 2008 [23] came with the hope and expectation that knowledge of the genomic sequences for these human malaria pathogens would both increase our understanding of the biology of these organisms and aid in development and deployment of interventions such as drugs and vaccines [24]. These original genome sequences have certainly increased our understanding of parasite biology, elucidating the basic genomic structure [22,23] and allowing comparisons between species to determine shared pathways among *Plasmodium* species that are unique to malaria. Functional genomics studies including investigation of transcription, proteomics, and metabolism have also provided key insight into the basic biology of the parasite. However, the promise of simply gleaning this genomic information for the development of effective drugs and vaccines has not been realized. Possibly the major advancement in the era of genomics applied to malaria is the realization that parasites from distinct geographical origins have different evolutionary histories shaped by their environment including the human host, the mosquito vector, and intervention strategies applied to those parasite populations including drug treatment [25]. This key observation implies that parasites can evade selective pressures either natural or applied and that patterns in genetic variation can recount this history. Thus, one can use knowledge about parasite genetic diversity to monitor parasite populations as transmission changes.

Transcriptomics

Post-genome transcriptome analysis of *P. falciparum* was initiated on a whole genome scale by two concurrent DNA microarray-based descriptions in 2003, which monitored transcriptome changes during the lifecycle of the parasite. A detailed investigation of the asexual intraerythrocytic developmental cycle (IDC) was performed by [26], delineating this 48 hour disease-causing cycle over intervals of 1h. Although different technologies and approaches were used by both groups, the overall conclusions were similar. The active transcription of the majority (>80%) of the predicted genes indicated a degree of overlap in the metabolic requirements during the different developmental stages of the parasite. However, 20% of all the predicted transcripts are IDC-specific and the high resolution of the [26] study indicated a periodic, 'just-in-time' nature of transcript production for >80% of these IDC transcripts. Genes are activated only once in a cascade manner, with peak expression just before the point at which their biological function is required [26].

Comparative transcriptomics of three geographically and phenotypically distinct strains of *P. falciparum* (3D7, Hb3, Dd2) characterized inter-strain differences [27] but remarkably, a high level of conservancy was found between these strains with significant transcriptional differences limited to genes encoding surface antigens. These results indicated a simple, highly conserved and streamlined cascade of gene expression.

More specialized reports described transcriptome analysis of the early stages of gametocytogenesis [28] and transcriptional features involved in the mechanism of var gene silencing, which influenced the regulation of genes distal to the var loci [29]. Comparison of wild type 3D7 *P. falciparum* to a gametocyte deficient clone with a

gene-specific custom array revealed differential expression of transcription factors and kinases that may play a role in the commitment to gametocytogenesis, the modulation of which may be used to block early gametocytogenesis and ultimately transmission [30].

To understand the underlying biology of the parasite while residing in the host environment, two studies described *in vivo* transcriptional profiling of *P. falciparum*. The high degree of correlation between the *in vitro* and *in vivo* transcriptomes was highlighted but over-expression of genes encoding a sexual stage antigen as well as gene families that encode surface proteins was shown [31,32]. Transcriptome analysis has also been utilized to understand the hosts' response to malaria infection. The response of Rhesus monkeys to *P. cynomolgi bastionella* infection was investigated using a human oligonucleotide chip [33] whereas a mouse oligonucleotide chip was used to investigate the effects of *P. berghei* infection. These studies showed that the expression of genes required for glycolysis, the host immune response, and erythropoiesis are modified, which correlates with the underlying pathology of disease progression.

Proteomics

The study of proteoms and their functions is crucial in the fight against malaria. Proteomic studies are essential to probe mechanistic changes and explain global protein expression profiles, differential protein expression, post-transcriptional control, post-translational regulation and modifications, alternative splicing and processing, subcellular localization, host–pathogen interactions, and the interconnectivity between these processes [34].

Some proteins uniquely expressed in a specific life stage have been identified through the use of proteomics. Proteomics analysis has revealed that a vast number of these gene products are not expressed in the host organism, which makes them promising as potential parasite-specific drug targets [5]. Proteomics analyses are also instrumental in validating predictions regarding the *Plasmodium* proteome generated using bioinformatics tools, and to further elucidate the mechanisms of action of currently used anti-malarial agents, such as quinolines and CoArtem. Given that proteomics is still a relatively new methodology with great potential for improvement, particularly regarding membrane spanning proteins, the prospects of providing new insight into the pathology and pharmacology of malaria through proteome analysis are indeed promising [5].

Genomic and transcriptomic studies have been very useful in investigating the expression profile in the genome predicting the function of uncharacterized genes [27]. However, questions remain in terms of conclusively identifying gene function, and current methods are dependent upon examining correlations in gene-expression profiles or co-regulation in transcription [35]. Similar limitations have been observed in proteomic studies, as a significant number of the identified proteins have unclear functions [36]. Therefore the acquisition of metabolomics data on parasite biochemical processes could provide an extremely useful complement to existing data sets and increase our ability to identify both gene and protein function [5].

Metabolomics

Metabolomics is a research approach that aims to identify and quantify the metabolome, which can be defined as the dynamic set of all small molecules present in a biological sample or organism under a given set of physiological or environmental conditions [37]. Metabolism is a key aspect of phenotype, and consequently the next logical step in functional genomics is to describe the distribution of metabolites in an organism following treatment or perturbation [38]. Given the complexities in studying the biology of the malaria parasite, metabolomic investigations could prove to be very elucidating. Metabolomics data are an important component of a systems biology analysis, especially when examining host–pathogen interactions. A prime target for a focused metabolomics study in malaria is lipid metabolism, which has been examined in a number of different reviews [39]. Ongoing drug development programs are exploring targets in lipid metabolism for development of antimalarials including *de novo* fatty acid synthesis [39], sphingolipid metabolism and lipid posttranslational modifications. It has been reported that parasites induce significant alterations in lipid parameters and that changes in lipid profiles occur in patients infected by protozoan parasites. In addition, lipid metabolism has been shown to be altered in the host by malaria infection and may reflect metabolic complications associated with severe malaria [40].

Metabolic pathway reconstruction can predict key enzymes and transporters that could be examined as potential new targets as well as reveal species-specific pathways that have an exploitable vulnerability [41] For example, the parasite appears to lack several proteins that in other eukaryotes are required for metabolism [42] and it is unable to synthesize several amino acids *de novo*. Similarly, metabolic pathways associated with the apicoplast organelle (a relict plastid (or chloroplast) derived from the endosymbiosis of cyanobacteria that is no longer photosynthetic) may potentially be targeted without interfering with host metabolism [43]. An omics approach to research questions will increase our ability to exploit these key differences in parasite–host interactions and increase our understanding of the biological processes involved, furthering our progress on the path to a permanent solution [44].

Application of genomics to transcriptomics, proteomics, and metabolomics

Genomic information has elucidated both transcriptional and epigenetic mechanisms of regulating gene expression using DNA microarrays [45] and RNA-Seq approaches. These studies reveal a highly ordered cascade of gene expression across the parasite lifecycle [26, 45]; that a major class of molecules regulating gene transcription are the AP2 (Apicomplexan Apetala2) proteins [46]; and that epigenetic regulation involves methylation that influences chromatin remodeling and var gene expression [47]. In a previous study, DNA microarrays identified a family of long non-coding RNA (lncRNA) molecules [48] proposed to play a key part of modulating parasite virulence by contributing to a mechanism of antigenic variation that allows parasites to evade host responses [49]. Transcriptional patterns have been interrogated directly from patients to help understand how parasites respond to host immunity. Toward illumination of patterns of immunity, protein microarrays based upon the genomic sequence are now being used to analyze host immune responses [50], which may help in understanding how the human immune system responds to infection and modulates disease. Similarly, genomics has helped identify novel pathways of parasite metabolism including the clever use of the carbon cycle that may be key to its survival in response to environmental conditions [51].

Advancements in technology-driven malaria diagnosis

The advancement in diagnostics can be grouped into two categories: technologies-driven and omics-driven. The advancements in technology-driven malaria diagnosis devices include microfluidics-based devices for cell-based diagnosis targeting hemozoin crystals in red blood cells (RBCs) [52], immuno-chromatographic tests (ICT) for the quantitative analysis of parasite protein (e.g., pfHRPII [53] or pLDH [54] (*P. falciparum* specific), pLDH (*P. vivax, P. ovale,* and *P. malariae*-pan) [55], aldolase (pan) [54] in the blood sample, 2-D paper matrix prototypes with dried reagents for quantitative analysis of parasite protein in the blood sample [56], and the DxBox 3-D plastic device [57] for differential diagnosis purposes. Spectrometry-based micro-Nuclear Magnetic Resonance (micro-NMR) [52], for diagnosing malaria, and mass spectrometry-based proteomics for host proteins [58] and parasite proteins have also shown promising results in the laboratory setup.

In parallel to the advances on the technology front, the development of different omics approaches has been explored to achieve ideal biomarker candidates [59]. Omics is a robust tool in terms of looking at a biological problem with varying points of reference, but to understand the bigger picture, one needs to combine the findings of various omics. Multi-omics (e.g., genomics, transcriptomics proteomics, metabolomics, or phenomics) or integrated omics reveal substantially novel insights into the pathobiology of chronic diseases [60], but remains a challenge in infectious diseases (e.g., malaria). While novel technologies are being developed, light microscopy remains the gold standard due to various challenges imposed by limited resources in rural areas.

Conclusion

Application of OMICS technologies in the fight against malaria will significantly advance our understanding of parasite biology and knowledge about parasite genetic diversity which can be used to aid discovery of vulnerable targets for intervention strategies, such as drugs, vaccines or insecticides, and to identify biomarkers for diagnosis, surveillance and monitoring of malaria infections in humans or mosquitoes. An omics approach to research questions will increase our ability to exploit key differences in parasite–host interactions and increase our understanding of the biological

processes involved, furthering our progress on the path to a permanent solution.

Acknowledgement

The author will like to acknowledge the contributions of Mr. Fada Malam, Mrs. Alhamdu J. T. and Mrs. Yakubu S.T. in the development of the initial draft of this manuscript.

Conflict of interests

The author declares no conflict of interests.

Funding source

None.

Ethical approval

Non-applicable.

Data availability

All data generated or analyzed during this study are included in this puplished article.

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