Omics: Applications related to diagnosis, treatment, prevention and control of parasitic diseases. Part II. Helminths

Review Article

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ABSTRACT

Landscape genomic studies can help define the role of geography and ecology in the clinical presentation of parasitic diseases, and consequently provide the choice for accurate diagnosis, novel therapeutic regimens, and effective strategies for control and elimination. In this regard, RNA interference technology combined with phenotypic studies can pinpoint exactly the incriminated molecules and/or pathways that might help in identification of novel drug targets and promising vaccine candidates. Previously, we discussed the applications of omics and bioinformatics in diagnosis, treatment, and control of malaria, one of the major health problems and causes of death worldwide[1]. In the present part, we intend to unravel the complexity of genomic and post genomic implications in the pathogenesis of helminthic diseases aiming to develop and design new therapeutic drugs and/or protective vaccines against helminths diseases.

Keywords: bioinformatics; drug target; helminths; omics; parasitic diseases; vaccine candidate.

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Abbreviations: AChE: Acetylcholinesterase; **E/S:** Excretory/secretory; **GST:** Glutathione S-transferase; **HSP:** Heat shock protein; **LC-MS/MS:** Liquid chromatography tandem mass spectrometry; **miRNA:** Micro RNA; **RNAi:** RNA interference.

***Schistosoma* spp.**

Host-parasite interaction: One mystery concerning the intra-mammalian life of *Schistosoma* adults is its adaptation to the anaerobic glucose metabolism and lactate production. This was explained lately by the presence of *Schistosoma* aquaporins that are membrane-bound proteins for water transport. Aquaporins capability to transport lactate across the cell membrane was also elucidated thus solving the mystery in *Schistosoma* adaptation and survival[2].

Identification of novel diagnostic biomarkers: Several micro RNA (miRNA) types were isolated from sera of patients with schistosomiasis, e.g. miR-124-3p, Bantam miRNA, miR-2C-3p, miR-3488 and miR2a-5p. Since miRNAs are secreted from *Schistosoma* extracellular vesicles, they are considered diagnostic biomarkers for diagnosis and therapeutic monitoring[3,4]. In 2022, advances in bioinformatics held a revolution in the diagnosis of schistosomiasis. Genomic datasets of *S. haematobium* and *S. japonicum* allowed the investigators to compare between both genomes utilizing gene ontology. Notably, the latter is a bioinformatics approach unifying genetic representation across a species followed by further *in silico* identification of identical proteins. Accordingly, distinct *S. japonicum* genes were demonstrated and enlisted as potential diagnostic markers for infection by Asian schistosomiasis[5]. Additionally, a study carried out in Nigeria demonstrated the role of bioinformatics in the design of *S. haematobium* diagnostic kit depending on *in silico* determination of high immunogenic *Schistosoma* proteins[6].

Identification of drug targets and vaccine candidates: Based on advances in schistosome genomics, a family of promising schistosome vaccine antigens were suggested. The best vaccine candidates identified against schistosomiasis are protein molecules expressed on the tegument surface or secreted by schistosomula such as *Sm*29, and *Sm*-p80 that attracted much attention. Notably, *Sm*29 is a glycosyl-phosphatidyl inositol (GPI)-anchored protein on *Schistosoma* tegument and the recombinant *Sm*29 vaccine exhibited Th1 immunity induction, and ~50% pathology reduction[7]. Besides, *Sm*-p80 is a subunit of the tegument cysteine protease, calpain that plays a crucial role in immune evasion. The recombinant *Sm*-p80 proved to be a promising vaccine candidate that achieved 80% improvement in liver pathology and a prominent Th1 response[8]. In addition to its role as a druggable molecule, investigating acetylcholinesterase (AChE) as a vaccine candidate against schistosomiasis was elucidated by omics and bioinformatics. Accordingly, two isoforms of AChE were identified in *S. mansoni*. The first was incorporated in the muscle layer to interact with heparin, i.e., a drug target. The other was GPI-anchored to the surface membrane, i.e., a potential vaccine candidate[9].

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Schistosomiasis is a complex disease regarding the pathogenesis of early clinical presentations up to the development of serious complications. One of the most important molecules that plays a pivotal role in *Schistosoma* development and endurance along all of its stages is the micro-RNA (miRNA). These non-coding small RNAs are indispensable for *Schistosoma* differentiation, tegmental development, nervous system functions, and apoptosis[10]. Later, a combined Chinese and Egyptian study was conducted to identify *Sj*-miR-124-3p in *S. japonicum* and its role in the parasite infectivity and development. The study utilized quantitative reverse transcriptomics to identify that *Sj*-miR-124-3p overexpression leads to de-arrangements in oogenesis, vitellogenesis, and tegument structure alterations, with subsequent decreased liver pathology. Accordingly, *Sj*miR-124-3p was proposed a promising vaccine candidate and potential therapeutic target (pivotal role)[11].

Discovery of novel drugs and vaccines: The role of *Schistosoma* glucose transporter 4 (SGTP4) was elucidated as pivotal for energy metabolism. Being a tegumental protein for host glucose uptake, Krautz-Peterson *et al*.[12] placed it in the druggable molecules list. Recently, a Brazilian study demonstrated the efficacy of eugenol derivatives in inhibiting *Schistosoma* transporter (Na^+/K^+ ATPase)[13].

Molecular docking, a computer based binding design, is a recent *in silico* technique for discovering therapeutic agents with high affinity to certain molecular ligands. This enhances the discovery of multi-drug targets to achieve ultimate therapeutic efficacy utilizing online library of compounds screening with minimum effort. Combined *in silico* molecular docking with *in vitro* studies was conducted to selectively detect highly effective potential drug targets (protein kinases) against schistosomiasis *mansoni*. The investigators proposed c-Jun N-terminal kinase (*Sm*JNK), extracellular signal regulated kinases (*Sm*ERK182)[14], and *Sm*P38[15]. Another identified *S. mansoni* protein kinase suggested as a promising drug target by RNA interference (RNAi) is tyrosine kinase (TK) feline sarcoma whose knockdown causes decreased schistosomal granuloma formation[16]. In another RNAi study, knockdown of genes encoding *Sm*JNK, *Sm*ERK182, and *Sm*P38 terminated parasite maturation and decreased parasite fecundity[17].

Several approaches were utilized to discover novel anti-schistosomal agents. Regarding repurposed drugs, Neves *et al*.[18] utilized a combined chemogenomic and bioinformatics approach. Antifungal, antibacterial, and other known drugs used in the treatment of cardiac and neurological diseases were identified as potential anti-schistosomal drugs. This study screened ~2000 proteins with cross-examination of their amino acid sequences by computational datasets. Griseofulvin, Gentamicin, Aprindine and Tetrabenazine were demonstrated targeting tubulin-β chain, heat shock protein 70 (HSP70), calmodulin, and vesicular transporters of *S. mansoni*, respectively[18]. Another strategy is the use of epigenetic probes/inhibitors. The probes constitute cell-active molecules that suppress specific domains in certain proteins, e.g., epigenetic controllers such as histone methyl transferase (HMT) and histone demethylase (HDM) inhibitor[19]. In such an approach, RNAi technology was utilized to identify the most likely druggable domain for these epigenetic probes/inhibitors. As demonstrated by vitellocyte inhibition, and egg production suppression, worm fecundity was the main deranged life aspect in *S. mansoni*. A third study used *in silico* approach for molecular docking experiments to determine the most potential druggable domains in epigenetic controllers. The investigators discovered that HDM inhibitor (GSK-J4) exhibited an anti-schistosomal activity through suppression of worm maturation, egg production and schistosomula transformation[20].

In a recent study in Philippines, investigators recognized *Sj*-8 and *Sj*4-1 proteins by IgG and IgE antibodies, in the sera of schistosomiasis *japonicum* patients after a whole proteome screening. Antibodies titers were significantly higher in individuals with resistance to reinfection compared to susceptible patients. The study further identified the *Sj*-8 and *Sj*4-1 localization that were excretory/secretory (E/S) antigens in the adults' tegument and gastrodermis. Utilizing RNAi, encoding genes silencing exhibited interference with worm nutrition rather than its fecundity, and decreased susceptibility of patients to reinfections as well. Accordingly, both antigens were suggested as promising vaccine candidates against schistosomiasis *japonicum*[21]. On the other hand, computational immunology involves the use of genomics and bioinformatics in the field of immunology. Immunoinformatics of *S. mansoni* heat shock proteins was utilized with *in silico* assessment of the antigenicity and physicochemical characteristics. The investigators succeeded to develop a 3D structured vaccine suitable for human use[22].

Peptidases, including cysteine proteases, have essential roles in several aspects for parasite survival, feeding, host invasion, and host immune evasion. *S. mansoni* cathepsin B (*Sm*Cath-B) is a cysteine protease present in the adult worm gut that degrades host's blood proteins. Moreover, RNAi technology showed that *Sm*Cath-B reduction exhibited growth impairment rendering the enzyme in the category of druggable molecules. In a recent study, Sudanese investigators designed novel SmCath-BI inhibitors utilizing *in silico* techniques, i.e., molecular docking, hybridization and dynamics[23].

Molecular docking studies identified active binding sites in *S. mansoni* purine nucleoside phosphorylase and *S. haematobium* 22 KDa glutathione S-transferase

(GST) to *Cucurbita maxima* pumpkin. Momordicoside I aglycone and Balsaminoside B were the two extracts with the maximum binding affinity to both parasitic drug targets. Hence, the two compounds were enlisted as potential anti-schistosomal agents[24]. Similarly, computer assisted studies and virtual screening pointed out several alkaloids with interesting schistosomicidal activities and plausible toxicity profiles. Alkaloids extracted from flowering plants, *Apocynaceae* and *Meninspermaceae*, showed best results[25].

Hepatic flukes

Host parasite interactions: It is obvious that bile-chemotaxis attracts *C. sinensis* juveniles into bile ducts where bile components stimulate their activity and growth. In the bile ducts, *C. sinensis* E/S products express molecules provoking pathologic changes in biliary epithelium with production of endogenous bioreactive radicles. Bioreactive radicals not only cause DNA damage, but also inhibit repair of host DNA damage promoting gene mutation. In fact, this phenomenon explains the high occurrence of cholangiocarcinoma in patients infected with *C. sinensis* and *O. viverrini*[26].

Vaccine candidates: Bioinformatics analysis and virtual screening combined with molecular docking approach allowed the investigators to elucidate the role of B-cell epitopes with high affinity receptors on the major histocompatibility complexes (MHC) I and II. A Turkey study developed a multi-epitope vaccine against fascioliasis that included B- and T-cell specific epitopes of four E/S antigens (a Kunitz-type endogenous serine protease inhibitor, cathepsin L1, helminth defense molecule, and GST)[27]. Additionally, *in silico* characterization and proteomic datasets of potential vaccine candidates in fascioliasis showed three of the newly discovered *Fasciola* Ly6s localized in extracellular vesicles, i.e., host/parasite interface. Notably, Ly6s family includes CD59-like proteins, homologues to CD59 human proteins with a proposed function of interrupting the host's complement system and thus suppressing the formation of the membrane attack complex[28].

***Echinococcus* spp.**

Host-parasite interactions: As it belongs to Platyhelminthes, *Echinococcus* spp. possess several revenues for host/parasite communication skills to achieve a successful modulation of host's immune response. Binding to parasite receptor tyrosine kinase for example leads to evoking signal molecules of host origin, mainly lipophilic hormones, that minimize host immunopathology. This explains parasite/host communication in parasite pathogenesis. One of the well-identified involved pathways is the initiation of the diverse cellular responses of tumor growth factor (TGF-β) through activation of receptor serine/threonine kinases. Phosphorylated receptor-activated Smads proteins are imported to the nucleus to regulate target gene expression[29]. This pathway was also linked to the subsequent activation of certain types of mitogen-activated protein kinase (MAPK) via activation of Smad-proteins leading to signal transduction of certain lipophilic hormones[30].

In 2012, Parkinson and colleagues performed a transcriptomic study to report the presence of the transmembranous tetraspanins proteins, in *E. granulosus* tegument. Tetraspanins were further localized in the protoscolices and adults. The study demonstrated that *Eg*TSP1 was necessary for protoscolices evagination and hence establishment of intestinal infections[31]. Later, short RNAi technology showed that silencing genes encoding *Eg*TSP1 consequently led to morphological tegument aberrations[32]. Other factors that determine host-parasite interactions in *Echinococcus* spp. include host insulin and host epidermal growth factor (EGF) that interact with parasite specific receptors to induce parasite kinases and moderate parasite growth[33,34].

Diagnosis: The *E. granulosus* P29 kDa antigen is a protoscolex-derived somatic antigen that was described mainly as a diagnostic biomarker for cystic echinococcosis. Its immunoinformatics analysis showed conservancy in all species and *Echinococcus* genotypes rendering it a reliable tool for screening and post therapeutic monitoring of hydatid cyst[35]. On the other hand, polymerase chain of *Ems*B microsatellite tandem repeats in the *Echinococcus* genome constitutes a method to increase the sensitivity of diagnosis. Several miRNAs were shown to be upregulated in the sera of echinococcosis patients especially those with alveolar disease. They proved to play an essential role in signaling inflammatory mediators, apoptosis, oxidative stress and macrophage proliferation[36].

Moreover, liquid chromatography tandem mass spectrometry (LC-MS/MS) proteomic analysis identified four *E. granulosus* histone variants. The differential distribution of histone variants reflected different parasite viability stages regarding their role in gene transcription and silencing and could interact with host cells. Bioinformatics tools enabled the investigators to characterize epitopes involved in antibody recognition. The three-dimensional structure of each histone variant was determined by virtual molecular screening, while PCR confirmed epitopes presence in the parasite genome[37]. Moreover, RNAi and microRNA knock-down recently contributed to the understanding of the cellular and molecular basis of tapeworm development and host-parasite interaction[38].

Discovery of novel drug targets: Results obtained from studies conducted on the nuclear genomes of *E. multilocularis* and *E. granulosus* were significant[39,40]. In addition to understanding parasite development mechanisms, recognition of several host-parasite interactions, and hence identifying novel drug

targets for development of new therapeutics was possible. Comparative analyses of the whole genome sequences of *E. multilocularis*, *E. granulosus*, *T. solium* and *Hymenolepis microstoma*, with comprehensive transcriptomic analyses of several *Echincoccus* developmental stages was performed. Both studies suggested several molecules as promising drug targets such as ion channels, proteases, G-protein-coupled receptors (GPCRs), and kinases because they were highly expressed in metacestode stage[39,40].

The high recurrence rates after treatment of alveolar echinococcosis were explained by genomic and transcriptomic studies. This recurrence was attributed to the resistance of the germinal layer cells to benzimidazoles due to the specific expression of benzimidazole insensitive b-tubulin isoform. Actually, the germinative cell is the main cell type responsible for the proliferation, differentiation, and regeneration of the parasite in the host. Hence, further drug design studies should focus on germinal cells. This identification of new drug targets will be facilitated by the transcriptomic analysis of the germinal cells by using gene expression patterns attained for hydroxyurea treated hydatid vesicles and parasite cultures[41,42].

The critical role of other proteins in the survival of *E. granulosus* different stages was identified by using short RNAi technology for specific gene silencing. These proteins include thioredoxin peroxidase[43] and calmodulin[44]. Calmodulin-specific short RNAi treatment was found to lead to disturbed contractility in *E. granulosus* proglottids, and consequent obstruction of worm survival[44]. The involved techniques include electroporation of primary cell culture. This powerful method studies gene function and regulation during early metacestode development. It is a transcriptomic approach for analyzing the effect of introducing oligonucleotides into the cells and probing the gene function loss[38]. Additionally, LC-MS/MS and *in silico* analysis of *Echinococcus* proteins revealed essential molecular functions related to pulmonary cystic echinococcosis[45]. These included adhesion, development regulation, enzymatic activity, and signaling transduction. This study also identified collagen, glycoproteins and laminin for extracellular matrix construction and function, in addition to tyrosine protein kinase and glypican-1 essential for signalling pathways; as well as identification of antigen B that is responsible for nutrient uptake of host lipids and metalloproteases and cysteine proteases for proteolytic activity. Accordingly, these drug targets were suggested for development of novel therapeutics to treat cystic echinococcosis and other larval tapewormes[45].

By applying a broad range of kinase inhibitors to *E. multilocularis* stem cell cultures, a proto-oncogene serine/threonine kinase was identified as a promising druggable substrate. However, repurposing of kinase inhibitors that was initially designed to affect mammalian kinases for helminths disease treatment is hindered by adverse side effects on human cells. The efficiency of high throughput *in silico* approaches to design small molecule compounds of higher specificity was studied with promising results[46].

Novel drug development: Interestingly, genomic analyses generated clear indications that cestodes employ a highly modified stem cell system[39,40]. Factors like Vasa and Piwi (classical germ cell markers in metazoans) are involved in preserving germline cells pluripotency in all bacteria and multipotency in somatic stem cells of many invertebrate lineages. These factors are absent in cestodes genomes. Although the associations of these modifications on stem cell maintenance and dynamics in cestodes are not yet clear[47], they could be related to the indefinite proliferation capacity that is typically observed in tapeworm larvae as *Echinococcus* or adults as *Taenia* spp. These findings brought the *Echinococcus* stem cell population (germinative cells) back into the focus of interest as a new therapeutic strategy[48,49].

Vaccines: Two studies carried out by Iranian researchers indicated that *Eg*P29 and cyclophilin could be the first step in the development of multiepitope vaccines against cystic echinococcosis[50,51]. The *Eg*P29 was tested *in silico* for the presence of B-cell and MHC epitopes that were determined and analysed. Several epitopes were marked using different servers and therefore *Eg*P29 was suggested as a multi-epitope vaccine candidate against cystic echinococcosis[50]. On the other hand, cyclophilins molecules present in many living organisms, are involved in the protein tertiary structuring. In the second study, Khazaei and Moghadamizad[51] demonstrated the potential of *E. granulosus* cyclophilin as a multi-epitope vaccine for cystic echinococcosis.

Neurocysticercosis (*T. solium*)

Development of novel drugs: Proteases are primary enzymes involved in parasites' host invasion by disrupting the physical barriers and immune system, i.e., promising drug targets. Calorimetric assay, LC-MS/MS and gelatin zymography were utilized to identify *Taenia* proteases and recognize their role in different metabolic processes. Drug docking studies to re-purpose the FDA-approved drugs showed 100% efficacy with a metalloprotease inhibitor that had low cytotoxicity in comparison to Praziquantel and Albendazole. Hence, it was proposed as a new drug for neurocystcercosis especially as it had better blood-brain permeability[52].

***Ascaris* spp.**

Drug resistance: Seven shared β-tubulin isotypes were identified in *A. lumbricoides* and *A. suum* genomes. Since benzimidazoles were expected to bind to all β-tubulin isotypes, the investigators utilized *in silico* docking study to demonstrate that the selection of

benzimidazoles to interact with one or two β-tubulin isotypes was likely the result of isotype expression levels affecting interaction frequency. Molecular dynamics simulations using β-tubulin isotype indicated that mutations of amino acids F200Y and E198A altered binding of benzimidazole[53].

Drug resistance: A recent study by Jones *et al*.[53] on *A. suum* and *A. lumbricoides* genomes revealed seven shared β-tubulin isotypes. Benzimidazoles were assumed to bind to all β-tubulin isotypes. Hence, an *in silico* docking study was attempted to show that the choice of benzimidazoles to interact with one or two β-tubulin isotypes, depended on the isotype expression levels. Molecular dynamics simulations using β-tubulin isotype, showed that mutation of amino acids E198A and F200Y changed the binding of benzimidazole[53].

Potential vaccine candidates: In another recent study[54], a reverse vaccine approach was used to predict new potential vaccine candidates. In this context, bioinformatics analysis of three Ascaris proteomes, chosen from the whole-genome sequences, identified candidate proteins. Notably, the bioinformatic tools tested the selected proteins for sub-cellular location, antigenicity, B-cell and T-cell molecular binding, and phylogenetic association with other nematode proteins. Trans-membrane proteins were suggested to be non-allergen antigens and could be used as promising vaccine candidates for ascariasis. These trans-membrane molecules were two voltage-dependent calcium channels, a protocadherin-like protein and a Piezo protein. The four proteins were expressed in ovaries or muscles of *Ascaris*. Additionally, they showed high affinity epitopes for B-cells and T-cells[54].

Strongyloides stercoralis

Diagnosis: Omics approaches were used for discovering a unique antigen specific for diagnosis of *S. stercoralis* either in serum[55] or stool samples[56]. In the first study, the investigators designed a multi-epitope antigen based on L3Nie.01 and IgG immunoreactive epitopes[55]. Utilizing phylogenetic comparison, the second study identified five specific coproantigens for diagnosis in stool samples, SCP/TAPS proteins family, transthyretin-like, AChE, aspartic peptidase, and prolyl oligopeptidase[56].

Brugia malayi

Potential drug targets: It is worth mentioning that the secretome, i.e., released organic and inorganic E/S molecules, was utilized to identify their essential role in cell adhesion, intercellular communication, morphogenesis, stage development, and immunomodulation. Accordingly, secretome targeting human hosts were detected by proteome-analyses and bioinformatics studies[57]. Metabolic pathway mapping and protease analysis of filarial worms revealed their unique secretome that differs from plant nematodes and entomophobes in the possession of pancreatic trypsin inhibitor, low density lipoprotein receptor, and tyrosinase-copper binding domain. These molecules render secretome a potential drug target[58]. Previously, it was reported that neuronal signaling was responsible for biological and functional actions through neurotransmitters in *B. malayi* as dopamine, biogenic amines and serotonin[59]. Besides, Cys-loop receptors on post synaptic nerves receive signals from presynaptic neurons by binding to the fore mentioned neurotransmitters, an action facilitated through ligand-gated ion channels. These Cys-loop receptors are now conceived as important drug targets[60].

The nuclear genome of *B. malayi* was identified as 95 Mb, besides a 14 kb mitochondrial genome and one Mb *Wolbachia* genome. Revolution in omics and bioinformatics led to better understanding of the worm behaviors and biology[61]. In fact, *Wolbachia* bacteria are indispensable for development and embryogenesis of the worm. They complement the nutritive needs of the nematode host as they retain the *de novo* synthesis capabilities for heme, riboflavin and nucleotides that are deficient in *B. malayi*. These bacteria represent an important drug target to control human filariasis[62].

Using the recent technologies of spatial transcriptomic analysis in *B. malayi,* the distribution of potential drug targets and vaccine candidates in the filarial worm was mapped. The openings on the parasite's body, especially the head region, were shown to be druggable sites and prominent locations for secretory antigens. These were located mainly on receptors in the region of the nerve ring and vulva[63]. The head region of the anterior female adult controls the behavioral and physiological functions of the helminth life[64]. This region, despite being small, accommodates the vital functions controllers of the adult *B. malayi*; including its reproduction, sensation, feeding and behaviors at the parasite-host interface. However, this part and its associated structures were undermined due to the focus on bulk transcriptomic and proteomic analysis of the whole worm substrates. An American study showed that the adult malayan filarial worm has an E/S system with a functional pore[63]. The study was mentored by spatial transcriptomics coupled with electron microscopy tissue capture techniques and RNA tomography. This facilitated specifying pivotal molecules in their spatial organization in tissues and thus a new era was opened in the discovery of druggable molecules that can be targeted in their specific tissues even in specific cells[63].

Recently, Flynn and colleagues[65] further investigated intestinal proteins as therapeutic agents for malayan filariasis. Transcriptomic analysis using short interference RNA inhibition identified an immunoglobulin superfamily cell adhesion molecule (IgSF CAH) as a pivotal molecule for worm motility, fecundity and derangement of the intestinal microvillus structure and pseudocoelomic fluid of the adult

nematode. The identified molecule was *Bm*-LAD-2 that was denoted as a druggable molecule. The investigators declared the absence of pre-existing IgE in the serum of infected patients, thus it was identified as a potential and safe drug target in endemic areas[65]. A homology was found between *Bm*-LAD-2 in *B. malayi* and L1 cell adhesion molecule in (L1CAM) of *C. elegans*. L1CAMs are transmembrane proteins important in signal transduction and in cytoskeletal integrity[66,67]. These CAMs in nematodes form cadherin-based junctions that are known for their crucial role in cell adhesion in eukaryotes[65]. In addition, loss of pseudocoelomic fluid into the intestinal lumen due to targeting *Bm*-LAD-2 proved the fore-mentioned role of the latter molecule in cell adhesion. It is known that this fluid constitutes the hydrostatic skeleton that confers the worm rigidity and effective movement. Loss of LICAMS can cause apoptosis and this explains the derangement in intestinal microvilli and the mitochondrial cristae of the adult *B. malayi* worms. Hence, it is evident that therapeutic targeting of junctional molecules in the intestine of *B. malayi* adult worms can alter the anatomy and subsequently the physiology and function of the alimentary tract; that is not only nutrition, but also metabolism, osmotic pressure regulation and waste disposal[65].

Finally, molting is a characteristic feature in filarial worms. Utilizing RNAi approach, a study determined 46 genes responsible for molting encoding proteases and DNA-binding and signaling proteins. A distinct molecular function characterized the cuticle of each life cycle stage through functional genomics of different types of cuticular collagen. Molting is controlled by a protein family called nuclear hormone receptors (NHR) represented by 27 genes in *B. malayi* genome[60].

Novel drug development: In *B. malayi*, the technology of molecular docking was used for identification of a natural molecule from a flowering plant (*Calotropis procera*) that binds with glutathione-s-transferase and plays a major role in detoxification of endogenous electrophilic compounds. This binding disturbs the detoxification process within *B. malayi*[68].

Wuchereria bancrofti

Vaccine candidate: Thioredoxin has the ability to cope with host oxidative challenges. A multi-epitope peptide-based vaccine used *W. bancrofti* thioredoxin in addition to epitopes of MHC-I, MHC-II, and toll-like receptors (TLRs) as specific adjuvants. Protein-protein molecular docking and simulation analysis between the vaccine and human TLRs was performed to investigate TLR5 role. In an *in silico* immune simulation experiment against *W. bancrofti*, TLR5 constituted the most potent receptor to transmit the vaccine-mediated signal that elicits an innate immune response with a satisfactory immunogenic report. This *in silico* study was used to construct a vaccine to target *W. bancrofti* thioredoxin for further *in vivo* studies[69].

Onchocerca volvolus

Diagnosis: Mass spectrometric analysis of *O. volvulus* crude extract revealed approximately 1400 proteins expressed in the adult and microfilariae. Computational analysis estimated six of the proteins as *O. volvulus* potential diagnostic markers. Linear epitopes were used to construct a multiepitope antigen (*Ov*MCBL02). Applications of this *Ov*MCBL02 antigen showed it is highly specific with no cross reactions with other nematodes[70].

Potential drug targets: An extracellular Cu/Zn-superoxide dismutase from *O. volvulus* (*Ov*EC-SOD) was cloned, purified and crystallized and proved to be an attractive drug target for onchocerciasis[71].

Trichinella spiralis

Host–parasite interaction: Secretome analysis of *T. spiralis* encysted larvae in muscles showed several E/S proteins that are considered as immunomodulators in the host. Nash *et al*.[72] showed that PouStich motif (one of the main pro-regulatory motifs) was associated with many proteins derived from *T. spiralis* stichosome oesophagus and seemed to master the function of the worm's secretome and facilitate the prediction of proteins secreted throughout *T. spiralis* lifecycle[72].

Novel drug development: The Cyt-domain of the progesterone receptor membrane component-2 of *T. spiralis* (PGRMC2-*Ts*) was identified, sequenced, cloned, and identified. Molecular docking studies showed that PGRMC2-*Ts* had different binding affinities with testosterone, dihydrotestosterone, progesterone, and estradiol. It was observed that progesterone affects *T. spiralis* oocytes by binding to PGRMC2-*Ts*. This information can reform the demographic criteria of susceptible individuals and elucidate the sex-predilection to trichinosis. Herein, it is worth mentioning that this study can impact deeply the trials to design new anti-parasitic drugs against trichinosis[73].

Proteomic analysis of the *T. spiralis* larval extract revealed 270 proteins. They were classified as cellular components, proteins involved in metabolic processes, and proteins with diverse biological functions. Analysis by the STRING database showed that most larval extract proteins were consistent and played critical roles in various metabolic processes. *In silico* analysis of anticancer peptides identified three candidates. Antitumor peptide 2 matched the hypothetical protein T01_4238 of *T. spiralis* and showed a diverse dose-dependent effect on the human liver cancer *HepG2* cell line, not by causing apoptosis or necrosis but by inducing ROS accumulation, leading to inhibition of cell proliferation[74].

Anisakis simplex

The E/S proteins of *A. simplex* L3 larvae were subjected to LC-MS/MS analysis and the identified

proteins were then analyzed using bioinformatics tools. In all detected ES proteins after detailed bioinformatic characterization, identified allergens were Anis 4 and 18. These are potential allergens, most of which are homologous to nematode and arthropod allergens. Nine potential pathogenicity-related proteins were also demonstrated, which were predominantly homologous to chaperones. In addition, predicted host-parasite interactions between the *Anisakis* ES proteins and both human and fish proteins were identified[75].

CONCLUDING REMARKS

1. *Schistosoma* miRNA are indispensable for understanding its system biology and host-parasite interactions. Omics studies revealed their potentiality in diagnosis and preference as drug targets.
2. Computational datasets facilitate drug repurposing, e.g., antibiotics, antimycotics and antipsychotics for use as anti-schistosomal agents, while molecular docking combined with RNAi technology constitutes a revolution in the discovery of novel anti-schistosmal drugs and vaccines.
3. Molecules, e.g., *Sm*P29 and *Sm*-acetylcholinesterase anchored to GPI-receptors are potential vaccine candidates.
4. The E/S products of *C. sinensis* showed bioreactive molecules that might complicate clonorchiasis with malignant cholangiocarcinoma.
5. In echinococcosis, RNAi assays proved that protein kinases are pivotal in parasite signal transduction and growth inside its host. Computational screening of protein molecules constitutes a leap in discovery of parasite druggable molecules and development of multi-epitope vaccines as *Eg*P29 and cyclophilin against cystic echinococcosis.
6. Drug docking revealed excellent efficacy of metalloprotease inhibitor as new therapeutic drug against neurocystcercosis.
7. *In silico* docking was utilized to demonstrate benzimidazoles selectivity to interact with one or two β-tubulin isotypes in *Ascaris* spp. The variability in their expression explains the changeability in drug sensitivity. Besides, proteomes predicted from whole-genome sequences were used to identify potential vaccine candidates.
8. Omics studies were also utilized to discover multi-epitope antigen, and five coproantigens for specific diagnosis of *S. stercoralis* in serum and stool specimens, respectively.
9. Omics and bioinformatics led to better understanding of the filarial worm behaviors and biology including *Wolbachia* essential roles with the discovery of new drug targets and vaccine candidates. Besides, the unique composition of filarial worm secretome renders it a potent druggable agent to combat lymphatic filariasis.
10. Mass spectrometric analysis of *O. volvulus* crude extract revealed six proteins as potential diagnostic markers. A multiepitope antigen (*Ov*MCBL02) was constructed to specifically diagnose onchocerciasis. Besides, Cu/Zn-superoxide dismutase from *O. volvulus* (*Ov*EC-SOD) proved to be an attractive drug target.
11. The PouStich motif derived from *T. spiralis* stichosome seems to master the function of *T. spiralis* secretome. Molecular docking of the progesterone receptor membrane component-2 (PGRMC2-*Ts*) elucidated the sex-prediction to trichinosis. *In silico* analysis of anticancer peptides identified three candidates in *T. spiralis* with new era in cancer therapy.
12. Proteomic analysis of *A. simplex* L3 revealed potential allergens that are homologos to nematode and arthropod allergens.

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