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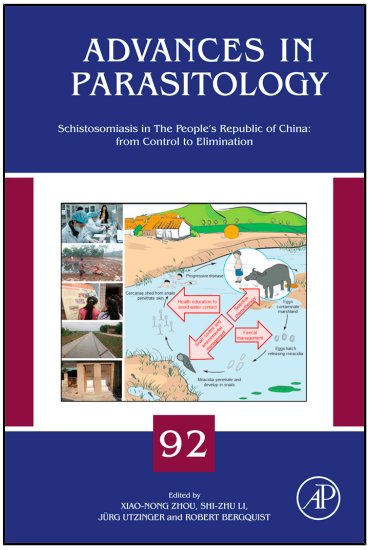

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CHAPTER THIRTEEN

New Anti-*Schistosoma* Approaches in The People's Republic of China: Development of Diagnostics, Vaccines and Other New Techniques Belonging to the 'Omics' Group

S.-B. Chen[*,§,¶], **L. Ai**[*,§,¶], **W. Hu**[*,§,¶,||], **J. Xu**[*,§,¶], **R. Bergquist**[#], **Z.-Q. Qin**[*,§,¶,1], **J.-H. Chen**[*,§,¶,1]

[*]National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai, The People's Republic of China
[§]Key Laboratory of Parasite and Vector Biology of the Chinese Ministry of Health, Shanghai, The People's Republic of China
[¶]WHO Collaborating Center for Tropical Diseases, Shanghai, The People's Republic of China
[||]Fudan University, Shanghai, The People's Republic of China
[#]Geospatial Health, University of Naples Federico II, Naples, Italy
[1]Corresponding authors: E-mail: junhuchen@hotmail.com; qinzq1008@hotmail.com

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Schistosomiasis in the Peoples Republic of China: from Control to Elimination, First Edition, 2016, 385–408

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Abstract

A new national schistosomiasis elimination programme will be implemented for the period 2016–20. To support this approach, we have performed a systematic review to assess anti-schistosome approaches in The People's Republic of China and defined research priorities for the coming years. A systematic search was conducted for articles published from January 2000 to March 2015 in international journals. Totally 410 references were published in English between 2000 and 2015 related to schistosomiasis after unrelated references and reviews or comments were further excluded. A set of research priorities has been identified for the near future that would improve the progress toward schistosomiasis elimination in The People's Republic of China. In particular, there is a lack of sensitive and specific tests for the detection of schistosomiasis cases with low parasite burdens, as well as an effective vaccine against schistosomiasis, and there is a need for surveillance tools that can evaluate the epidemic status for guiding the elimination strategy. Hence, we think that schistosomiasis control and elimination will be improved in The People's Republic of China through development of new tools.

1. INTRODUCTION

The recent publication of the genome of *Schistosoma haematobium* represents a useful addition to the previously released genomes and transcriptomes of *Schistosoma mansoni* and *Schistosoma japonicum* (Berriman et al., 2009; Hu et al., 2003; Neafsey et al., 2012; Protasio et al., 2012; Consortium, S.j.G.S.a.F.A., 2009; Verjovski-Almeida et al., 2003). The unravelling of the genomes of the three schistosome species that case the great majority of human infection has provided research opportunities on many aspects of schistosome biology. This genomic information is an extremely valuable resource for global postgenomic research activities, such as DNA microarray profiling, proteomics, immunomics, metabolomics and glycomics that make up the core of the 'omics' group of disciplines (Hokke et al., 2007; Lier et al., 2009). Although this approach to the study

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of schistosome biology is still in its initial stage, there are important technological advances, not only for new diagnostics, and vaccines, but also for our understanding of disease mechanisms, host–parasite interactions and transmission biology based on the genomic resources of the different schistosome species (Brindley et al., 2009; Lustigman et al., 2012).

Screening of potential vaccine and diagnostic antigens and development of new anti-schistosome tools by high-throughput proteogenomic approaches remain a research priority (Chen et al., 2014; Gaze et al., 2014). Firstly, in spite of the existence of the safe and highly effective drug praziquantel (PZQ) for schistosomiasis treatment, disease transmission cannot be control by chemotherapy alone, eg, mass treatment does not prevent reinfection, and the potential risk of parasite resistance is a potential threat when mass drug administration is repeated for the long term (Tambo et al., 2015). Therefore, vaccination strategies represent an important supplementary component alongside chemotherapy for sustainable control of schistosomiasis (Hotez et al., 2010; McManus and Loukas, 2008). Secondly, most of the currently available diagnostic antibody-detection techniques, based on whole-schistosome crude extracts, as have low specificity and poor reproducibility (Zhang et al., 2015). Thus, improved direct, diagnostic tests with high specificity and sensitivity, are required to determine transmission interruption and elimination of schistosomiasis following control efforts (You and McManus, 2015; Zheng et al., 2013). Thirdly, the availability of functional genomic tools for investigating the schistosome biology, eg, RNA interference (RNAi) (Lustigman et al., 2012) is still limited. Control of schistosomiasis in The People's Republic of China requires more than chemotherapy alone and preferably new approaches based on omics, such as new vaccines and other new techniques in the future (Chen et al., 2012). The aim of this the review is to identify new tools under development and inform about which anti-schistosome tools are already now available.

2. MATERIAL AND METHODS

2.1 Search strategies and selection criteria

A systematic search was conducted for articles published from January 2000 to March 2015 in international journals in the Medline database through PubMed, the database of the National Library of Medicine in the United States. We used the following search criteria, ie, (schistosomiasis

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japonica or *S. japonicum* [Title/Abstract]) AND The People's Republic of China AND English [Language] AND ('2000/01/01' [Date − Create]: '2015/03/31' [Date − Create]) (Fig. 1). The references were exported from PubMed into Endnote X1 (Thompson Reuters, San Francisco, USA), duplicates were removed and the file was transferred to Excel 2007 (Microsoft Corp, Seattle, USA).

Each article was assigned to at least one of the following subjects based on the keywords included in the reference: omics (genomics, RNAomics, proteomics, transcriptomics, metabolomics, immunomics, glycomics), vaccines, new techniques (molecular diagnosis, immunodiagnosis, genotyping, crystal structure, RNAi). We excluded the following articles: review, education, software, comment, book, health promotion and commercial-related topics.

2.2 Statistical analysis

Data were processed using Excel (Microsoft, WA, USA) filters. Excel was also used to compute index analysis for references published in PubMed.

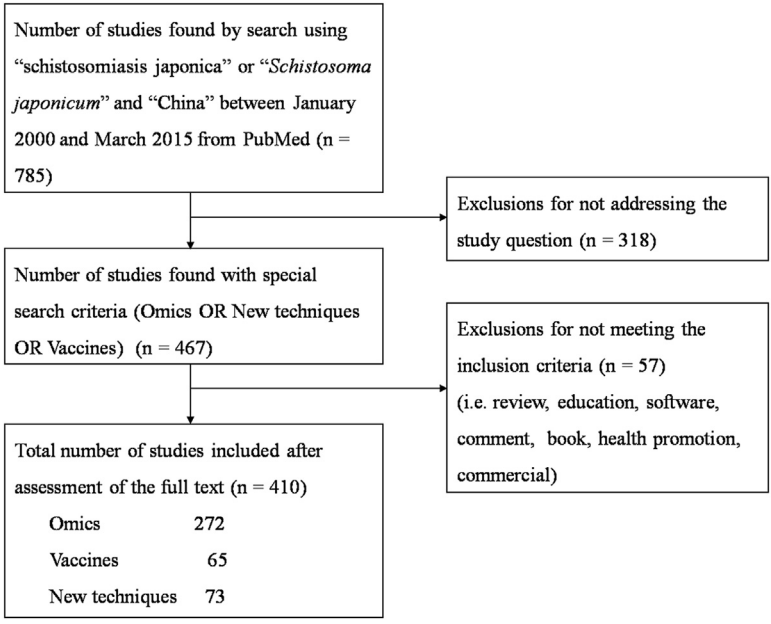

Figure 1 ***Flowchart visualizing the procedure for identifying relevant articles.*** Articles about 'schistosomiasis japonica' or *Schistosoma japonicum* from January 2000 to March 2015 in international journals from PubMed (n = 785). Articles did not address the study question were excluded from the search results (n = 318); review, education, software, comment, book, health promotion, commercial were also excluded (n = 57). Remaining articles were categorized according to subject, including omics (n = 272), vaccines (n = 65), new techniques (n = 73).

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3. CURRENT RESEARCH STATE

3.1 General information

An exponential increase of references published in PubMed ($R^2 = 0.730$) was observed for the period January 2000 to March 2015 (Fig. 2).

Fourteen journals published 10 or more articles each on *S. japonicum* research in The People's Republic of China during January 2000 and March 2015 (Table 1). *Parasitology Research* and *Acta Tropica* are the top two journals for publication of *S. japonicum* research in The People's Republic of China. In addition, relevant works from Chinese researchers were also published in *New England Journal of Medicine* (Wang et al., 2009), *Lancet* (Wang et al., 2008), *Lancet Infectious Diseases* (Wang et al., 2008), *Nature* (Consortium, S.j.G.S.a.F.A., 2009), *Nature Genetics* (Hu et al., 2003).

Fifteen academic and research organizations in The People's Republic of China published 10 or more articles on *S. japonicum* research searched from PubMed from January 2000 to March 2015 (Table 2). National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (The People's Republic of China CDC) is the topest research institution for publication of *S. japonicum* research in The People's Republic of China.

3.2 Omics

In total, 272 references were found for −omics of *S. japonicum* in The People's Republic of China from PubMed with English language after unrelated references and reviews or comments were excluded from the search results.

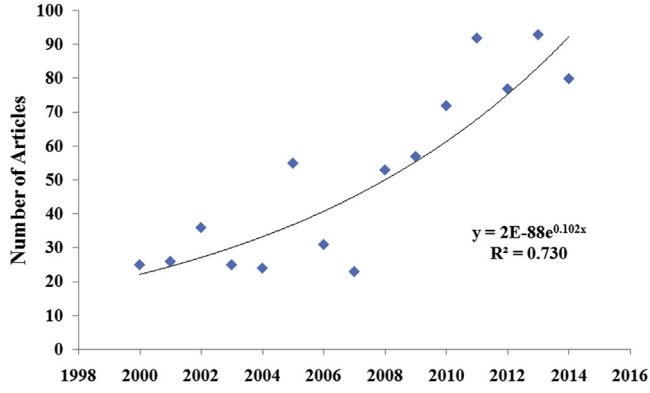

Figure 2 Distribution of references for *Schistosoma japonicum* research in The People's Republic of China in PubMed.

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Table 1 Journals having published 10 or more articles on *Schistosoma japonicum* research in The People's Republic of China (PubMed search)

Journal	Articles
Parasitology Research	106
Acta Tropica	70
Parasites and Vectors	39
PLoS One	39
PLoS Neglected Tropical Diseases	36
Parasitology International	26
Experimental Parasitology	25
American Journal of Tropical Medicine and Hygiene	23
International Journal of Parasitology	23
Parasitology	23
Southeast Asian Journal of Tropical Medicine and Public Health	21
Annals of Tropical Medicine and Parasitology	14
Vaccine	14
Transactions of the Royal Society of Tropical Medicine and Hygiene	10

Table 2 Fifteen institutes in The People's Republic of China published 10 or more articles on *Schistosoma japonicum* research

Institution	Articles
National Institute of Parasitic Disease, China CDC	80
Jiangsu Institute of Parasitic Disease	53
Huazhong University of Science and Technology	48
Nanjing Medical University	47
Shanghai Veterinary Research Institute, CAAS	47
Fudan University	29
Central South University	28
Wuhan University	23
Sun Yat-sen University	22
Anhui Medical University	19
Hunan Institute of Parasitic Diseases	14
Tongji University	14
Soochow University	13
Chinese Academy of Medical Sciences & Peking Union Medical College	13
Guangzhou Medical University	10

CAAS, Chinese Academy of Agricultural Sciences.

The remaining references were categorized according to subject, including genomics (18.38%), RNAomics (4.78%), transcriptomics (8.09%), proteomics (39.34%), immunomics (26.47%), metabolomics (2.57%) and

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glycomics (0.37%) (Fig. 3). Systems biology and functional genomics aim to integrate 'omic' information for a better understanding of cellular biology.

3.2.1 Genomics

The genome of *S. japonicum* was deciphered and published in 2009 under the efforts from Chinese researchers (Consortium, S.j.G.S.a.F.A., 2009). The genome of *S. japonicum* (397 Mb) has eight chromosomes, including seven pairs of autosomal chromosomes and one pair of ZW type sexual chromosomes and encompass 13,469 protein-coding genes.

3.2.2 RNAomics

Unlike messenger RNAs (mRNAs), small noncoding RNAs (sncRNAs) represent a group of nontranslatable transcripts which are approximately 18–30 nucleotides in length and serve as critical regulators to silence or activate specific target genes in fungi, plants and metazoans (Bartel, 2009; Brennecke et al., 2007; Molnar et al., 2010). Three main components of sncRNAs, small interfering RNAs (siRNAs), microRNAs (miRNAs) and Piwi-interacting RNAs (piRNAs) have been well established and extensively studied (Kim, 2005). Using protocols similar to conventional transcriptomic research, which can be outlined as RNA isolation, library construction and sequencing (Cheng and Jin, 2012), vast numbers of schistosomal siRNAs and miRNAs have been successfully detected in

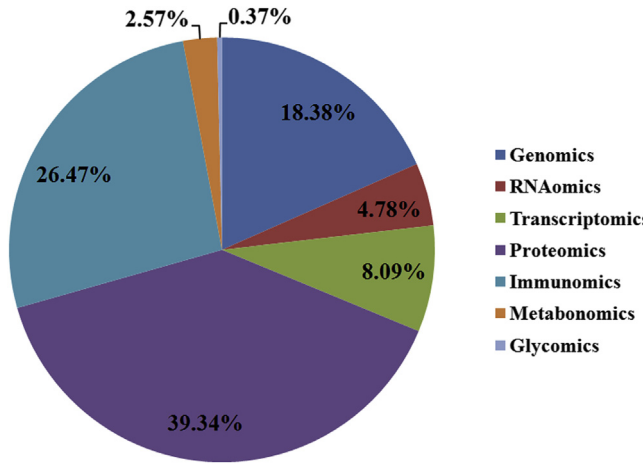

Figure 3 Percentage distribution of references for omics on *Schistosoma japonicum* in The People's Republic of China.

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S. japonicum by Chinese researchers (Dai et al., 2014; Hao et al., 2010; Wu et al., 2014; Xue et al., 2008).

3.2.3 Proteomics

Gene expression is the creation of a product (most often a protein) based on the transfer of information from a gene. This process involves several steps and relationship between the transcriptional and the translational level is not always straightforward. Proteomic analysis plays an important role as it aiming at the characterization and comparison of functions, abundance and subcellular localization of numerous gene products derived from single or multiple samples (Chuan et al., 2010). Taking a schistosomal specimen as example, it would first be separated by one-dimensional gel electrophoresis (1DGE) or 2DGE, subsequently subjecting isolated bands or spots to digestion followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Wang et al., 2013) or liquid chromatography–tandem mass spectrometry (LC-MS/MS) (Cao et al., 2015) depending on the method of separation chosen. This process would eventually provide information on the specimen's protein identities through by database searches (van Hellemond et al., 2007). Heretofore, schistosomal proteomics has been applied to the investigation and comparison of protein compositions in various developmental stages and between the two genders (Cheng et al., 2005; Liu et al., 2009, 2014)

3.2.4 Transcripomics

The continuing release of updated and new helminth, genome sequences has catalysed the emergence of transcriptomic analyses, which are providing unprecedented approach to understanding biological function that should eventually result in novel control interventions. *Schistosoma japonicum* transcripomics research commenced from the establishment of complementary DNA (cDNA) expression libraries, which has generated expressed sequence tags (ESTs) from nearly all stages of *S. japonicum* (Peng et al., 2003). After the numerous ESTs data have been obtained, microarray and serial analysis of gene expression technology have been applied to profile schistosome transcripts of from different stages and/or under distinct conditions. These applications can be classified into four main categories: (1) characterization of individual cell/tissue types; (2) profilation of intact organisms and life cycles (Chai et al., 2006); (3) study of parasite–host interactions and effect of chemotherapy (You et al., 2009) and (4) study of gene expression differences between geographical isolates or species.

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3.2.5 Metabolomics

Metabolomics is the study of metabolites that appear in response to outside influences, eg, infections. Thus, each infection produces specific, cellular processes that reveal themselves as unique chemical fingerprints that can be used for profiling. For example, such a strategy was used to investigate the metabolic responses of Syrian hamsters (SLAC) experimentally infected with *S. japonicum* using various spectroscopic methods, eg, high resolution ^{1}H nuclear magnetic resonance spectroscopy, MS and capillary electrophoresis, coupled with various data-mining technologies. Several metabolites related to schistosome infection have been profiled, eg, in urine samples from hamsters infected with *S. japonicum* (Wang et al., 2006), as well as in blood and multiple tissue samples from rodents coinfected with *S. japonicum* and *Necator americanus*) (Wu et al., 2010).

3.2.6 Immunomics

Immunomics is a study that combines proteomics with serology and aims at ascertaining the interaction between host immune system and exotic antigens after pathogen invasion. Early schistosomal immunomics utilized combined 2D Western blotting and MS to identify and compare proteins recognized in serum samples from patients exposed for *S. japonicum* cercariae before and after PZQ treatment or testing the situation at different ages and infection intensities. Utilizing immunomics together with a protein microarray has also been applied in an attempt to seek prospective vaccine targets (Driguez et al., 2010). This advanced technology consists of several steps including gene selection, cell-free expression, chip printing and serological probing (Chen et al., 2010, 2014, 2015; Fan et al., 2013). An immunomics approach to schistosome antigen discovery was used in seeking antibody signatures in naturally resistant and chronically infected individuals from endemic areas (Gaze et al., 2014). Screening diagnostic candidates for schistosomiasis from tegument proteins of adult *S. japonicum* using an immunoproteomic approach has also been attempted (Zhang et al., 2015).

3.2.7 Glycomics

The name glycomics, formed to follow the convention established by genomics, proteomics, etc., refers to systematic study of glycan structures, including genetic, physiologic and pathologic aspects. It has been well accepted that glycans play critical role in the induction of innate immune responses during schistosome–host interplay, the profiling of schistosomal

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protein- and/or lipid-conjugated glycan or free glycan elements was addressed even before the screening of schistosomal transcriptomics and proteomics. However, it is not clear what kind of worm glycans can affect the infected host to mount IgG responses and whether mounted anti-glycan IgG responses are protective. The research group from The People's Republic of China discovered that both periodate-resistant periodate-sensitive glycans are antigenic, and dominant anti-glycan IgG responses can play important roles in protective immunity in schistosome-infected host (Gong et al., 2015).

3.3 Schistosomiasis vaccine development in The People's Republic of China

Although PZQ is current cornerstone of schistosomiasis control strategy, there is a need to find a complementary approach that can provide a more long-term prevention. The solution would be immunization with an anti-schistosome vaccine combined with PZQ treatment (Bergquist et al., 2005).

Some of the leading *S. japonicum* vaccine candidates, the protective efficacies of which in different host animals are summarized in Table 3, are discussed later.

Table 3 The current *Schistosoma japonicum* vaccine candidates

Schistosome antigen (protein or cDNA)	Experimental animal (worm reduction)		References
	Mouse	Domestic animals (pig, water buffalo)	
Attenuated carcarie	33–77%	80–90%	Lin et al. (2011)
Attenuated carcarie		89%	Shi et al. (1993)
Adult worm SjGST 26–28	26–32%	25–69%	Wei et al. (2008)
Adult worm Sj31–32	28%		Wang et al. (2008)
Adult worm SjTPI[a]	21–25%		Dai et al. (2014)
Adult worm SjTPI[a] (i.m.)[b]	50%		Dai et al. (2014)
Adult worm Sj23	27–35%	32–59%	Wang et al. (2014)
Mimotope peptide[c]	26–32%		Wang et al. (2005), Wu et al. (2006)

Some of the leading *S. japonicum* vaccine candidates are discussed, the protective efficacies of these and other molecules in different host animals are summarized.
[a]Triosephosphate isomerase.
[b]Intramuscular injection.
[c]Recombinant Sj338 protein.

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3.3.1 Live attenuated vaccines

The initial approach to the development of a schistosome vaccine in The People's Republic of China relied predominantly on live attenuated cercarial vaccines. Cercariae and schistosomula, attenuated in various ways (gamma rays, X-rays, ultraviolet irradiation), have been reported to elicit high levels of protective immunity against schistosomiasis. Irradiated schistosome cercariae confer protection against infection in experimental animal challenge models. For example, water buffaloes vaccinated with irradiation-attenuated *S. japonicum* cercariae, developed 89% resistance to reinfection (Shi et al., 1993). In addition, Chinese researchers have reported that high levels of protective efficacy (77.62%, 88.8% and 99.78% reduction in worm burden, liver eggs and faecal eggs, respectively) against *S. japonicum* challenge were obtained with an ultraviolet-attenuated cercarial vaccine in pigs (Lin et al., 2011). The killing mechanism might be mediated through effective IFN-γ response and strong antibody-mediated response (Lin et al., 2011). Live vaccines are suitable for inducing protection in the laboratory but are not considered for vaccinating humans or domestic animals in the field. The reasons for this include: (1) production costs and labour-intensive efforts required to obtain large numbers of cercariae from infected *Oncomelania* snails; (2) the difficulties in standardizing the dose of ionizing radiation to induce cercarial attenuation and (3) the potential toxicity of administering a live vaccine. To circumvent these problems, Chinese scientists have attempted to improve the protection expressed by the attenuated cercarial vaccines by substituting chemically defined schistosomal antigens genetically engineered in bacteria or yeasts or virus. To date, promising results have been obtained with three major classes of recombinant vaccines, ie, plasmid DNA vaccines, mimotope-based peptide vaccines and vaccines based on viral vectors.

3.3.2 Plasmid DNA vaccines

DNA vaccines use a plasmid containing the gene(s) that code for an immunogenic protein(s) of interest (Zhang et al., 2015). Until recently, no licensed DNA vaccine is commercially available; however, this technology has gained considerable attention, and several products are at various developmental or experimental trial stages, eg, a recombinant plasmid DNA vaccine, pVAX1/SjTGR that is currently being tested in BALB/c mice. The recombinant plasmid, administrated by a 'gene gun', has achieved an average protective efficacy assessed as 27.8–38.8% worm reduction and 40.4–44.5% of liver egg count reduction. All animals vaccinated with pVAX1/SjTGR developed significant specific anti-SjTGR antibodies. Moreover,

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animals immunized by the gene gun exhibited a splenocyte proliferative response, with an increase in IFN-γ and IL-4 cytokines (Cao et al., 2013). Purified vaccine antigens are often poorly immunogenic, even when produced as recombinant subunit vaccines, and require additional components, such as adjuvants, to stimulate protective immunity based on antibodies and effector-T cells. Adjuvants are currently widely used in animal models, but they are rarely used in human. Immune stimulation by cytokines has been successful in the control of *S. japonicum* infection of poultry as experimental animal (Wei et al., 2008). For example, enhancement of a vaccine-induced immune response was achieved by co-administration of a DNA vaccine encoding for *S. japonicum* 14 kDa fatty acid binding protein (SjFABP) or *S. japonicum* 26 kDa GST with IL-18 (Wei et al., 2009). In both cases, significant increase in the humoral immune responses has been reported. In another study, cimetidine (CIM), a histamine-2-receptor antagonist, as an adjuvant, with pEGFP-Sj26GST (the recombinant plasmid containing enhanced green fluorescent protein gene and the gene encoding 26 kDa glutathione S-transferase of *S. japonicum*) DNA vaccine to immunize mice. The results showed a reduction of both worm and egg burdens in the pEGFP-Sj26GST + CIM group (79.0% and 68.4%, respectively), significantly higher than that in pEGFP-Sj26GST alone group (27.0% and 22.5%, respectively). Compared with the pEGFP-Sj26GST alone group, mice immunized with pEGFP-Sj26GST plus CIM showed an elevated level of IFN-γ and IL-12 and a low level of IL-10 in splenocytes, while the levels of IL-4 and IL-5 showed no difference between the two groups (Li et al., 2011). Wang X reported that by using levamisole (LMS) as an adjuvant enhances cell-mediated immunity in DNA vaccination; VR1012-SjGST-32, in an LMS formulation in the murine challenge model. Compared to controls, the VR1012-SjGST-32 plus LMS reduced worm and egg burdens, as well as, the associated immunopathological complications significantly in liver, apparently associated with a Th1-type response. Together, these results suggest that the LMS as a potential schistosome DNA vaccine adjuvant can be useful for prevention, possibly mediated through the induction of a strong Th1-response (Chen et al., 2012). DNA vaccines have some limitations, since most DNA vaccines are administered by injection, which makes their application difficult in large commercial application. APAMAM dendrimers as a novel vaccine delivery vector will assist in protecting the vaccine against enzymatic degradation and may enhance the immunoreactivity of DNA vaccine and increase the protective effect of the SjC23DNA vaccine against *S. japonicum* infection (Wang et al., 2014).

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3.3.3 Mimotope-based peptide vaccines

One interesting approach toward the discovery of new antigens with biotechnological applications is based on phage display technology (Clark and March, 2004). Phage display has been used to identify mimotopes (peptides that mimic linear, discontinuous, and even nonpeptide epitopes) to be employed as vaccine candidates (Arnon et al., 2000). The mimotope peptide vaccines are derived from pathogen protein or a portion of the genome coding for immunogenic epitope. A study has demonstrated that in an effort to identify protective epitopes in Sj338 and increase the level of protection in murine vaccination all four of these phages demonstrated 34.2% worm reduction compared to controls vaccinated with the M13 clone (Xu et al., 2007). Although at experimental stages of development, mimotope peptide vaccines have been shown to be promising for schistosomiasis control, some researchers have focused on developing multiepitope peptide vaccines, eg, clones from a phage library were selected by innate resistance antibodies present in sera samples of rats and *Microtus fortis*, respectively. Expression analyses and immunization study using the screened and enriched mimotope peptides yielded significant humoral and cell-mediated immune responses that resulted in >30% protection after challenge with *S. japonicum* cercariae (Ouyang et al., 2003; Wang et al., 2005). These data suggest that mimotope peptides are potential vaccine candidates.

3.3.4 Viral vector-based vaccines

The use of viral vectors to deliver gene(s) of interest has been studied extensively. Remarkably, the ability of adenovirus vector to persist in cells without causing pathology as well as their tropism to various dividing and nondividing cells allows sustained antigen release. It is also possible to package and express different immunogenic protein subunits in vector-based vaccines without the necessary use of a whole virulent organism. Experimental recombinant vector vaccines have been developed against *S. japonicum*. For example, intramuscular rAdV-SjTPI.opt (triosephosphate isomerase from *S. japonicum* (SjTPI)) provided a consistent and repeatable higher protective effect in mice (more than 50%). These findings may be due to the associated higher levels of specific Th1, antibody responses and partially lower level of IL-17A (Dai et al., 2014). Similarly, Hu have constructed a modified adenoviral vector system that may serve as an alternative delivery vehicle for schistosome vaccine development (Hu et al., 2014).

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3.4 New techniques

In total, 73 references were found for new techniques of *S. japonicum* in The People's Republic of China from PubMed with English language after unrelated references and reviews or comments were excluded from the search results. The remaining references were categorized according to subject, including immunodiagnosis (63.01%), molecular diagnosis (16.43%), RNAi (10.96%), genotyping (5.48%) and crystal structure (4.11%) (Fig. 4).

3.4.1 Immunodiagnosis

Immunodiagnostic techniques have a high sensitivity, are easy to perform and are an excellent epidemiological tool for the screening of target populations in schistosome-endemic areas (Zhang et al., 2016). Many variations of indirect immunodiagnostic assays in schistosomiasis diagnosis include the circumoval precipitin test and indirect haemagglutination assay (IHA) which have been widely used historically, and dipstick dye immunoassay and enzyme-linked immonosorbent assay (ELISA), using soluble egg antigen (SEA) as the source of target antigen, is the most widely used technique currently (You and McManus, 2015; Zhu, 2005). Diagnosis based on the detection of antibodies is very sensitive and relatively repeatable, tractable and time-saving compared with traditional direct parasitological methods. Early indirect immunodiagnosis relied on the crude extracts of worm components, like microsomal extract, gut-associated polysaccharide, nonfractionated extracts of eggs, etc. However, early

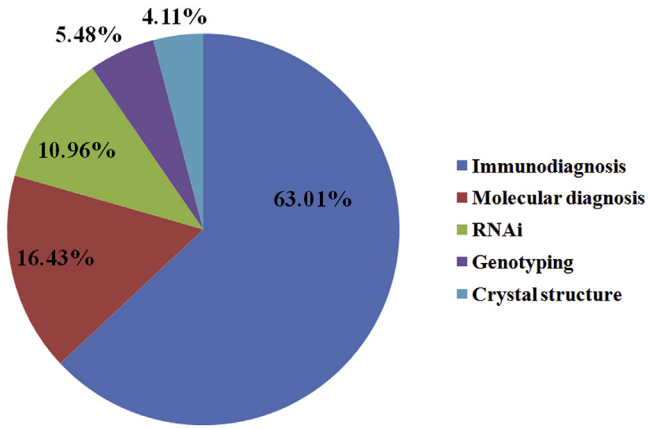

Figure 4 Percentage distribution of references for new techniques of *Schistosoma japonicum* in The People's Republic of China.

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indirect immunodiagnosis relied on the crude extracts of worm components including microsomal extract, gut-associated polysaccharide and nonfractionated extracts of eggs, which faces a cross-reaction problem (Jin et al., 2010). Furthermore, antibody-based serological assays are not quantitative and usually fail to discriminate between previous exposure and current infection. Although it is difficult to avoid innate drawbacks like these, these diagnostic techniques could be improved to some extent by the use of selected recombinant antigens. Owing to the enormous amount of gene sequence information procured by the genomics and transcriptomics research, together with screening methods including proteomics, immunomics as well as some bioinformatics tools, many promising diagnostic antigens have been identified and their immunogenicity have been successfully tested (Zhang et al., 2009, 2015), vastly enriching the diagnostic antigen tool box.

Transcriptomic resources have provided cDNA expression libraries. Combined serological screening and immunological verification, schistosomal antigens with high immunogenicity could be identified in cDNA expression libraries and finally be applied for antibody-based immunodiagnosis of schistosomiasis (Zhou et al., 2010) used rabbit sera collected on day 21 postinfection in an antigenic screening of a previously constructed cercariae cDNA expression library of *S. japonicum* (Fung et al., 2002). Likewise, another group of Chinese researchers (Zhou et al., 2009) used human saliva instead of rabbit sera to screen a cDNA expression library of *S. japonicum* eggs, which resulted in the detection of the Sj13 antigen. The following ELISA assay exhibited 92.5% sensitivity and 92.1% specificity of salivary IgG detection with recombinant Sj13. Given the easy-accessibility and noninvasiveness of the saliva samples, saliva/Sj13 was asserted as a promising alternative to serological test for schistosomiasis. In addition, numerous antigens sorted from the cDNA expression library established in 2003 (Hu et al., 2003), which including 43,707 ESTs derived from adult *S. japonicum* and their eggs. Furthermore, bioinformatics techniques also served as screening tools to seek antigen candidates for indirect immunological diagnosis in the *S. japonicum* genome. In another study, immunomics was also employed to uncover prospective diagnosis antigens for schistosomiasis (Zhong et al., 2010).

Early immunoassays have shown that schistosome-derived antigens, such as adult worm antigen (AWA), SEA and various circulating antigens (Zhao et al., 2012) are released into the host circulatory system/excreta in infected humans and other hosts, which facilitates the research on direct immunological diagnosis of schistosomiasis. Circulating anodic antigen

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(CAA) and circulating cathodic antigen are by far the most extensively used antigens for the antigen-based immunodiagnosis of schistosomiasis and methods for the detection of circulating antigens encompass sandwich ELISA, IHA, time-resolved immunofluorometric assay, magnetic bead immunoassay, reagent strips and liquid-phase piezoelectric immunosensor. Compared with the two foregoing diagnostic methods, the detection of circulating antigens is more suitable for drug efficacy trials owing to its high specificity and the ability to discriminate between previous exposure and current infection. The significantly higher prevalence of active schistosome infections by the urine and serum upconverting phosphor lateral-flow CAA assays has implications for the national control and elimination programme, particularly in respect to case-finding and intervention strategies while the downward trend in prevalence and intensity of *S. japonicum* infection in The People's Republic of China has reached a level (van Dam et al., 2015). It is important to mention this here as this technique is in fact more sensitive than the molecular techniques since they target antigens emitted from the worm in the blood, while the latter approach generally targets egg antigens leaving the body with the faeces.

3.4.2 Molecular diagnosis

Given the existence of *S. japonicum* DNA in host serum derived from dead schistosomula, tegument shedding of worm or inactive eggs (Xu et al., 2013), molecular diagnosis based on the polymerase chain reaction (PCR) has become a promising alternative to overcome the innate shortcomings of etiological diagnosis and immunodiagnosis for schistosomiasis. Since the publication of the usefulness of the PCR for detecting *S. mansoni* DNA in human faecal and serum sample (Pontes et al., 2002), PCR-based molecular diagnosis has been applied to the detection of schistosomes in multiple diagnostic field works and several novel technologies, such as real-time PCR (RT-PCR), PCR-ELISA and loop-mediated isothermal amplification (Tambo et al., 2014; Xu et al., 2010), etc. have been added to the tool box in addition to conventional PCR for boosting the detection sensitivity. Empirical evidence shows that molecular diagnosis of schistosomiasis has both high sensitivity and high specificity, enabling detection during the larval stage or at least before eggs are released in the host, and can use various materials, eg, faeces, serum, plasma and urine as samples. These reasons suffice to convince users that they are superior to conventional diagnostic approaches. Factors that impact the result of molecular diagnosis are various and the choice of amplified products is definitely among them. However,

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confined by the inaccessibility to the schistosomal genome sequence, in the pregenomic era, researchers could only select PCR targets with limited candidates.

A large-scale selection had been conducted to seek suitable target sequences among 25 retrotransposons identified along with the publication of the genome of *S. japonicum* to achieve a specific and sensitive diagnosis (Consortium, S.j.G.S.a.F.A., 2009; Guo et al., 2012). To begin with, primer pairs were designed for the 25 candidates with *Sj*R2, a previously described molecular diagnostic target, serving as positive control. A series of diluted genomic *S. japonicum* DNA were used as templates in a PCR assay to amplify the target fragments and judge the sensitivity of those 25 candidates. The result showed that in addition to *Sj*R2, a new non-LTR retrotransposon named *Sj*CHGCS19, with the PCR product of 303-bp in length, had high sensitivity for detecting *S. japonicum* DNA. Coincidentally, bioinformatics research showed that both *Sj*CHGCS19 and *Sj*R2 have higher genome proportions, repetitive complete copies and partial copies and more active ESTs compared with other candidates in the *S. japonicum* genome, which may also outline the general features of ideal targets for PCR diagnosis of schistosomiasis.

Parasite-derived miRNA strands, similar to genomic DNA, are also present in host biofluids, eg, plasma, serum, urine and saliva, which support their potential as biomarkers for the molecular diagnosis of schistosomiasis. Besides, in response to the invasion of pathogens, some characteristic changes of the host's miRNA profile might appear, which can also serve as a hint of an infection event. Nowadays, the available sequences of schistosomal miRNAs, in association with those approaches established for the profiling of miRNAs, eg, stem-loop RT-PCR, miRNA array and high-throughput sequencing have paved the way for the use of miRNA-based molecular diagnosis of schistosomiasis in the future.

3.4.3 RNA interference

RNAi is a potent gene silencing process triggered by exogenous double-stranded (ds) RNA. Knockdown of genes using RNAi has proven to be a powerful technique for investigating a variety of gene functions in schistosomes (Britton et al., 2012; Krautz-Peterson et al., 2010). Schistosomes undergo many morphological and physiological transformations during development as a result of profound changes in gene expression when moving from one stage of the life cycle to the next. Proteins containing zinc finger (ZF) motifs usually play an important role in DNA

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recognition, RNA packaging and transcriptional activation. In a study, Liu et al. (2015) cloned the open reading frame of *S. japonicum* SjZFP1, which encodes a ZF protein, analysed the cDNA sequence of SjZFP1 and examined the expression of SjZFP1 mRNA from various developmental stages. This research group also tested the effects of RNAi with respect to worm burden, spawning and egg hatching. The results suggest that the SjZFP1 gene might be important for parasite development and egg production in the vertebrate host, as well as egg hatching in the water.

The worms survive in the capillary networks in the abdomen of the final host, where thioredoxin glutathione reductase (TGR) activity helps them to survive in the aerobic environment (Wang et al., 2014) synthesized four small interfering RNAs (siRNA S1, S2, S3 and S4) using them to target and knock out the SjTGR gene. The knockout effect of the siRNAs resulting in closing down the thioredoxin reductase activity of SjTGR were evaluated in vitro. The results of this study indicate that SjTGR may play an important role in the clearance of oxygen free radicals and protection of *S. japonicum* parasites against oxidative damage.

3.4.4 Genotyping

In the present study, intersimple sequence repeats (ISSRs) markers were used to examine the genetic variability of *S. japonicum* isolates from different provinces in The People's Republic of China, using *S. japonicum* from Japan and *S. mansoni* from Puerto Rico for comparison. Of the 30 primers screened, 4 produced highly reproducible ISSR fragments. Using these primers, 107 discernible DNA fragments were generated with 105 (98.13%) being polymorphic, indicating considerable genetic variation among the examined *S. japonicum* isolates. The percentage of polymorphic bands among *S. japonicum* isolates from The People's Republic of China and Japan was 82.2%, 43.9% among mountainous type isolates and 64.5% among lake/marshland type isolates from The People's Republic of China. Unweighted pair group method analysis revealed that all of the *S. japonicum* samples were grouped into two clades, the first containing Chinese isolates and the other Japanese ones. Within the cluster of *S. japonicum* isolates from The People's Republic of China, isolates from mountainous Sichuan and Yunnan provinces grouped together, whereas isolates from lake/marshland regions (Anhui, Jiangsu and Hubei provinces) clustered together. The results demonstrated that the ISSR markers are useful for studying genetic diversity and population structure of *S. japonicum* isolates from The People's Republic of China (Wang and Li, 1990; Zhao et al., 2009).

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3.4.5 Crystal structure

Thioredoxin-glutathione system of *S. japonicum* plays a critical role in maintaining the redox balance in parasite, which is a potential target for development of novel anti-schistosomal agents. Researchers cloned the gene of *S. japonicum* thioredoxin (SjTrx) from cDNA, expressed and purified the recombinant SjTrx in *Escherichia coli*. Functional assay shows that SjTrx catalyses the dithiothreitol reduction of insulin disulphide bonds. The coupling assay of SjTrx with its endogenous reductase, TGR from *S. japonicum* (SjTGR), supports its biological function to maintain the redox homoeostasis in the cell. Furthermore, the crystal structure of SjTrx in the oxidized state was determined at 2.0 Å resolution, revealing a typical architecture of thioredoxin fold. The structural information of SjTrx provides us important clues for understanding the maintenance function of redox homoeostasis in *S. japonicum* and pathogenesis of this chronic disease (Alicino et al., 2015).

4. CONCLUSIONS AND RECOMMENDATIONS

An exponentially increasing number of references published in both of English and Chinese from PubMed was observed by our systematic search for published articles on *S. japonicum* research covering the 2000–15 timeframe. In total 410 references were published in English. Articles on omics were the largest proportion of articles published in English during the latest 15 years.

Although we think that schistosomiasis control and elimination will be improved in The People's Republic of China through development of new tools facilitating the march toward schistosomiasis control and elimination in The People's Republic of China, a series of research gaps were identified that could hinder progress (Zheng et al., 2013). For example, there is a lack of sensitive and specific tests for the detection of schistosomiasis cases with low intensity of infection, and there is a need for a vaccine that can reduce symptoms and slow transmission. Hence, They need to be regarded as one component, albeit a very important one, of integrated schistosomiasis control programs that complement existing strategies.

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