

Minireview

Epigenetic markers associated with schistosomiasis

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Summary

It is important to consider the use of the epigenome as source of complementary data for genome knowledge, which is suitable for the diagnosis of schistosomiasis. Usually, a laboratory diagnosis of schistosomiasis is performed by means of **1.** Egg detection in the stool or urine by microscopy remains with limited sensitivity; **2.** Immunological screening, in which positivity persists after treatment, and **3.** Molecular appraisals prevail over the disadvantages of the currently used methods. In this sense, molecular methodologies are being developed based on epigenetic biomarkers, aiming to improve the diagnosis of the disease and clinical treatment as early as possible to prevent the occurrence of serious liver damage.

**Keywords:** Schistosomiasis; DNA methylation; histone modification; miRNAs; epigenetic markers

Introduction

Schistosomiasis is caused by helminths of the genus *Schistosoma*, which comprises approximately twenty species, six of which are capable of infecting humans. *Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum* are the main causative agents of intestinal and urogenital schistosomiasis, which represent the second most prevalent parasitic disease in the world (Weerakoon *et al.*, 2015). Schistosomiasis is one of the 17 neglected tropical diseases (NTDs) prevalent in people and communities living in poverty and social exclusion. This disease reaches around 260 million people worldwide; of these, more than 100 million are children aged 5 to 14 years (Weerakoon *et al.*, 2015). Due to the great social and economic impact of schistosomiasis, the World Health Organization established the year 2025 as a limit for its control or eradication (WHO, 2013). The current problems of schistosomiasis involve factors such as complexity of the *Schistosoma sp.* life cycle, low drug efficacy, lack of effective

vaccines, and limited access to potable water and sanitation in endemic areas. The extent of schistosomiasis is contained to specific areas; its transmission depends on the presence of a susceptible intermediate host, such as snails *Biomphalaria ssp.*, *Bulinus ssp.*, *Oncomelania ssp.*, water conditions, the migration process of people, and anthropogenic activities that cause environmental changes (Colley *et al.*, 2014; Steinmann *et al.*, 2006).

The most effective strategy to control schistosomiasis transmission involves the construction of sewage networks, water treatment, adequate intermediary host elimination strategies, health education, diagnosis, and treatment of infected people (Molehin *et al.*, 2016). Many countries, including Brazil, adopted plans to control schistosomiasis, especially those from less developed regions. However, despite great efforts, the actions employed are still inefficient and schistosomiasis remains a public health issue (Ross *et al.*, 2014).

Efforts have been made to aid in the identification of drug targets and vaccines, the development of diagnoses and chromosome

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maps as well as the arrangement and analysis of the genome sequence; for this reason, studies related to the expression of *S. mansoni* genetic material were initiated in the 90's, with the *S. mansoni* Genome Project (Loverde *et al.*, 2004; Zerlotini *et al.*, 2010). These studies were carried out by researchers from Universidade Federal de Minas Gerais (UFMG), Centro de pesquisas René Rachou, Fundação Oswaldo Cruz (FIOCRUZ), and with a special contribution of Dr. Craig Venter of the Institute for Genomic Research (IGR), USA. During the period from 1994 to 2000, more than 17,000 expressed sequence tags were identified by means of contributions of Schistosoma Genome Network researchers, supported by the WHO (Franco *et al.*, 1997; Oliveira *et al.*, 2007). This number of potentially expressed tags increased to over 200.000 sequences by 2003 with the publication of the *S. mansoni* transcriptome initiative conducted by a group of researchers in São Paulo state and international collaborators (Verjovski-Almeida *et al.*, 2003). Following these studies, the *S. mansoni* genome was characterized as consisting of seven pairs of autosomal genes and one sexual pair (male ZZ; female ZW) (Zerlotini *et al.*, 2010). In addition, it was established that the genome sequences consist

of 380 megabases, covering approximately 12,000 genes that encode (on a provisional basis) different profiles of stage-specific transcriptomes (Anderson *et al.*, 2015; Berriman *et al.*, 2009; Cai *et al.*, 2016).

From studies on the genetics of *S. mansoni*, it was established that the parasites influence host cells by altering both chromatin and DNA. These changes were denominated as epigenetic alterations, which refer to hereditary changes in gene expression that remain stable after some cellular divisions and are not mediated by changes in the DNA sequence (De Oliveira *et al.*, 2017; Roquis *et al.*, 2015). The hereditary marks provide regulatory functions distinct from changes in the base pair sequence of the genome (Stewart-Morgan *et al.*, 2020). Both the genome and the epigenome are influenced by internal and external factors, which interact with each other to determine hereditary effects on the phenotype of individuals, thus leading to differences in genetically identical individuals (Rivera & Ren, 2013; Shah *et al.*, 2014). The variation of phenotypic characteristics by the action of epigenetic factors, which irreversibly affect gene expression, is an intriguing question for researchers (Weerakoon *et al.*, 2015). The epigenome

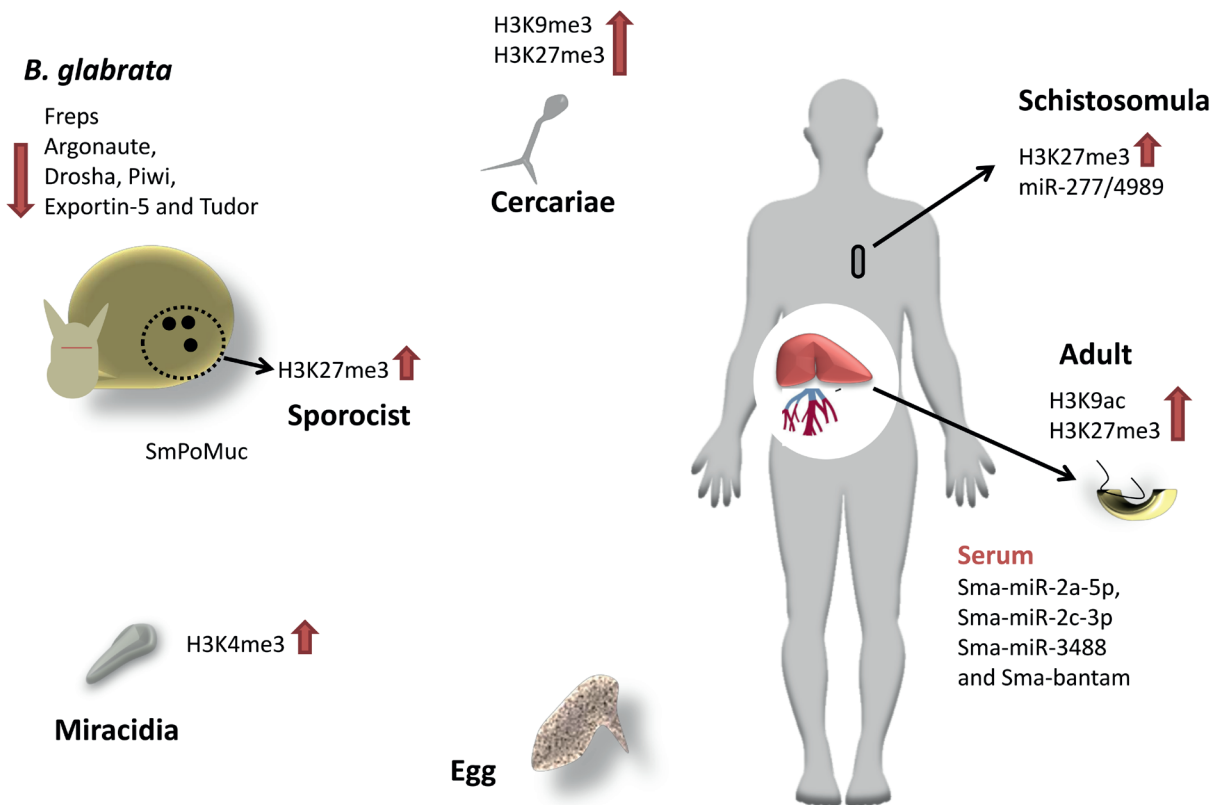


Fig. 1. Epigenetic regulation throughout the *S. mansoni* cycle: The figure shows some epigenetic marks enriched in the different stages of *S. mansoni* and some miRNAs involved in parasite-host interaction. Larval stage mucins (SmPoMuc) interact with snail immunity proteins. The intermediate host has downregulation of several miRNA and piRNA biogenesis pathway proteins during miracidium penetration and also during the subsequent stages of development. The most enriched epigenetic marks in cercariae are characteristics of gene silencing. In schistosomes, miRNAs 277 and 4989 may be related to adult worm development. In adults there is an enrichment of epigenetic marks associated with eucromatization. There are miRNAs from the parasite that have already been found in human serum and have been proposed as possible diagnostic biomarkers.

integrates a set of aspects or phenotypes; being characterized by a dynamic and pliable response to either intra and extracellular stimuli caused by interaction with adjacent cells, exogenous molecules, physiological mechanisms and/or by the environment to which the organism is exposed. The resulted epigenetic changes play an important role in the cell evolution because they are transmitted by more than one generation (Anderson *et al.*, 2012; Shah *et al.*, 2014). Therefore, the epigenome integrates information encoded in the genome with all molecular and chemical signals from cellular, extracellular, and environmental origins (Rivera *et al.*, 2013). Epigenetic mechanisms are essential for the regulation of gene expression and involve a large number of modifications such as DNA methylation in CpG residues and its demethylation, histone tail modifications, and non-coding RNAs (Berdasco *et al.*, 2018).

From the sequencing of the *S. mansoni* genome, studies on epigenetic mechanisms involved in the development and differentiation of the schistosome have been better understood and are likely to play a crucial role in the transformations occurring at all stages of the Schistosome life cycle; these involve strict control of gene transcription (Cabezas-Cruz *et al.*, 2014). Due to the plasticity of the genome, the life cycle of *S. mansoni* involves a complex differentiation process, to guarantee its survival under different conditions of environmental stress during its free-living phase and at the physiological conditions of a definitive host (Roquis *et al.*, 2016), (Fig. 1).

The spectrum of interactions between the genome and the epigenome of the parasite and the hosts are marked by the presence of epigenetic markers that play important roles in regulatory processes. The aim of this review is therefore to evaluate the main epigenetic factors that can be considered possible epigenetic biomarkers of mansoni schistosomiasis.

### **Molecular epigenetic markers associated to Schistosomiasis**

A biomarker can be defined as a “marker that reflects or integrates one or more biological activities” that can be detectable and measured (Berdasco *et al.*, 2018). Using biomarkers, it is possible to predict the onset and course of a disease, or to evaluate the patient’s response to a specific treatment (Berdasco *et al.*, 2018). The use of biomarkers for diagnosis must consider the characteristics by which a particular disease can be identified and monitored. The assay methods must be widely applicable for most individuals and populations, and they also should present sensitivity and specificity to generate a correct disease diagnosis (Berdasco *et al.*, 2018). In this context, genomic sequences related to the main species of *Schistosoma*, allied to new technologies, constitute a pathway for the identification of biomarkers and methods of diagnosis, treatment, and control of human schistosomiasis. Methylation epigenetic marks in histones can act as good biomarkers and therapeutic targets. As an example, in *S. mansoni* larval stages of miracidia, sporocyst and schistosomula were detected predomi-

nance of non-constitutive chromatin activated mark, H3K4me3 (trimethyl-histone H3 (Lys4)), in TSS (Transcription start site). Cercariae contained bivalent marks, H3K4me3 and H3K27me3 also known as trimethyl-histone H3 (Lys27), that indicates a transitory stage of gene activation and repression in TSS. The same study described an equivalent presence of both three marks in adult worms, nevertheless the repression mark was located upstream and the others at TSS (Roquis *et al.*, 2018). Epigenetic marks as biomarkers are already applied to cancer treatment aiming to trace a personalized therapy based on epialleles profile (Smith *et al.*, 2020). Considering *S. mansoni* parasites this methodology could be useful in a comparative way among the stages, detecting where are the genes activated in adult and therefore key therapeutic targets. A comparison of epigenetic landscape between susceptible and resistant *S. mansoni* strains could suggest alterations induced by resistance and thus would be possible to outline a treatment to humans infected with resistant strains in the future.

An example of epigenetic writers is the expression of SmCBP1 (Creb-binding protein 1) and SmGCN5 (General control non-repressed 5) that is related to the normal development of eggs and the reproductive system of females. These histone acetyltransferases are recruited by nuclear receptor heterodimer SmRXR1/SmCBP1 that binds to the *Smp14* promoter. SmCBP1 and SmGCN5 leads the histone modification and chromatin decondensation increasing the transcription of that gene. The inhibition of these histone acetyltransferases (HATs) by both RNAi and an enzyme inhibitor in females causes malformation of the eggs, such as abnormal lateral spine, discontinuous or fissured shell and invaginations. In addition, the females have a reduced ovary (Carneiro *et al.*, 2014). This proves that these epigenetic writers could be used as a potential preventive target of schistosomiasis, because eggshell formation is an essential step in egg development; furthermore, viability and egg release are necessary for the transmission and immunopathology of this disease (Carneiro *et al.*, 2009; Carneiro *et al.*, 2014).

The serum of mice and humans infected with *S. mansoni* has elevated quantities of HMGB1 (High mobility group box 1) protein. It is reported that this protein induces several pro-inflammatory cytokines augmenting the hepatic fibrosis process besides inducing the expression of  $\alpha$ -SMA (Alfa-smooth muscle actin) in Hepatic Stellate Cells. The inhibition of host’s HMGB1 shrinks granuloma areas (Vicentino *et al.*, 2018). Other studies described HMGB1 encoded by *S. mansoni* and this ortholog, secreted by parasite eggs, also generates inflammatory immune responses. An important fact linked to this protein is related to the presence of lysine sites potentially acetylated by both SmCBP and SmGCN5 (Carneiro *et al.*, 2009). The traffic of human HMGB1 to cytoplasm is mediated by acetylation, but in *S. mansoni* its traffic does not depends on acetyl posttranslational modification. HMGB1 was initially described as a chromosomal protein, important in chromatin structure maintenance and remodeling and also as a transcriptional activator of some specific genes, functioning as an epigenetic reader (De

Table 1. Main enzymes and reactions involved in epigenetic processes in *S. mansoni*.

Enzyme type	Reaction catalyzed	Reaction example	Access code or reference
DNMT2	DNA methylation	Cytosine + S-Adenosyl-L-methionine DNA- methyltransferase Cytosine-CH <sub>3</sub>	(Geyer <i>et al.</i> , 2011) Smp_198180
HDACs	Histone deacetylation	H <sub>2</sub> O + N <sup>ε</sup> -acetyl-L-lysyl-[histone] = acetate + L-lysyl-[histone]	<i>SmHDAC1</i> Smp_005210; <i>SmHDAC3</i> Smp_093280; <i>SmHDAC8</i> Smp_091990; (Oger <i>et al.</i> , 2008)
HAT	Catalyzes acetylation in lysines of histones and other proteins	Zinc ion binding Bromodomain Acetyltransferase activity	AY337317 Smp_105910 (Bertin <i>et al.</i> , 2006; Maciel <i>et al.</i> , 2004)
HMT PRMT	Protein arginine N-methyltransferase 1 S-adenosyl-L-methionine Methyltransferase . Peptidyl-arginine N-methylation	Catalyze the formation of omega-N monomethylarginine (MMA) and symmetrical dimethylarginine (sDMA).	Smp_070340 Smp_029240.2 Smp_025550 Smp_171150 Cabezas-Cruz <i>et al.</i> , 2014
PTKs	Phosphorylation of tyrosine protein diversity	ATP + a protein-L-tyrosine ADP + a protein-L-tyrosine phosphate	(Avelar <i>et al.</i> , 2011)
Ubiquitin- conjugating enzymes (Ub-E2)	Catalyzes the intermediate step of protein ubiquitination	S-ubiquitinyl-[E1 ubiquitin-activating enzyme]-L-cysteine + [E2 ubiquitin- conjugating enzyme]-L-cysteine = [E1 ubiquitin-activating enzyme]-L- cysteine + S-ubiquitinyl-[E2 ubiquitin- conjugating enzyme]-L-cysteine.	(Costa <i>et al.</i> , 2015)
UCHs, USPs, OTUs, MJDS	Involved in ubiquitin processing, in the recovery of modified ubiquitin trapped in inactive forms, and in the recycling of ubiquitin monomers from polyubiquitinated chains DNA repair, cell-cycle control, endocytosis, transcription and protein degradation by the proteasome	Thiol-dependent hydrolysis of ester, thioester, amide, peptide and isopeptide bonds formed by the C-terminal Glycine of ubiquitin	(Pereira <i>et al.</i> , 2015)
HMT PKMT	Protein lysine methyltransferase (PKMT)	L-lysyl-[protein] + S-adenosyl-L- methionine = H <sup>+</sup> + N <sup>ε</sup> -methyl-L-lysyl- [protein] + S-adenosyl-L-homocysteine	(Smp _000700) (Whatley <i>et al.</i> , 2019)
KDM	Lysine specific demethylase	Demethylation of 'Lys-27' of H3 and pendant methylation of 'Lys-4' of histone H3, in agreement with recruitment of the PRC1 complex and monoubiquitination of histone H2A	(Smp_034000) (Lobo-Silva <i>et al.</i> , 2020)

DNMTs = DNA-methyl-transferase, Ub-E2 = ubiquitin-protein conjugation, HAT = Histone acetyltransferase, HDAC = Histone deacetylase MJDS = Machado-Joseph disease proteases, OTUs = ovarian tumor proteases, PTKs = Protein tyrosine kinases, UCHs = Ubiquitin C-terminal hydrolases, USPs = ubiquitin-specific proteases

Abreu *et al.*, 2016). HMGB1 exhibits promising characteristics of a diagnostic biomarker due to its secretion in sera and of prognosis also due to its different localization profiles during the acute and chronic schistosomiasis (Carneiro *et al.*, 2009; Vicentino *et al.*, 2018).

Among the main non-canonical epigenetic mechanisms microRNAs (miRNAs) have also been implicated in the course of schistosomiasis. Studies have described miRNAs derived from the parasite in the host's serum or derived mainly from the host's liver differentially expressed in the serum during infection by *S. mansoni* (Meningher *et al.*, 2017). These authors consider that the host's miRNAs are not good biomarkers of schistosomiasis, because in other pathologies that affect the liver such as cirrhosis, hepatitis and cancer. They also present their altered expression profile, in this case they are markers of liver fibrosis or damage (Meningher *et al.*, 2017; Hoy *et al.*, 2014). In this context, detecting parasite derived biomarkers is a more efficient way to achieve specificity in the diagnosis. Epigenetic biomarkers would be advantageous in monosexual infections, in which Kato Katz would be impracticable, and also to detect schistosomiasis in patients infected with resistant strains, since the oviposition of resistant worms is quite reduced in relation to susceptible ones (Abou-El-Naga *et al.*, 2019), in addition to being possible to identify some epigenetic biomarkers already in the schistosomule stage, which remains undetectable in routine tests.

Epigenetic markers are produced through interactions between the parasite and the environment, as well as by interactions with host-specific immune responses. Therefore, to use epigenetic factors as biomarkers, the relationships between parasite and its definitive host must be considered (Mota *et al.*, 2020). In epigenetic processes where the interactions occur by modifying the epigenetic state, there is the participation of enzymes such as: DNA methyltransferases (DNMT), protein arginine methyltransferase (PRMT), protein lysine methyltransferase (PKMT), lysine demethylases (KDM), protein lysine acetyltransferase, protein lysine deacetylase (HDAC), kinases, ubiquitin conjugating enzymes (E2), deubiquitinating enzymes (DUBs), and others, presented in Table 1.

Other types of diseases that are also associated to epigenetic pattern alterations, such as miRNA expression, DNA methylation, histone modifications, and others, reinforce the study of epigenetics (Sandoval *et al.*, 2013).

The inflammatory processes observed in mansoni schistosomiasis involves a typical granulomatous lesion formed around the eggs deposited by the parasite. The egg has a squamous epithelium constituting an internal protective membrane called the "von Lichtenberg envelope", which is synthetically active and capable of producing molecules with antigenic properties known as soluble egg antigens (SEAs) (Klaver *et al.*, 2015). When released, the SEAs cross tissues inducing a granulomatous reaction, which is considered late-type hypersensitivity. During the acute phase, the reaction is exacerbated, and the granuloma reaches a volume up to 100 times that of the egg considering rodents as definitive hosts.

In the chronic phase, this granuloma regresses, reaching smaller dimensions (Chuah *et al.*, 2016). During this biological cycle stage, histone acetylation involving HATs is an important mechanism; they enable the expression of egg proteins and the maturation of ovaries in *S. mansoni*. The inhibition of HATs leads to malformation of the reproductive organs and, consequently, to fragile eggs (Carneiro *et al.*, 2014).

In this context, several findings related to epigenetics involved in biological processes and differentiation stages of *S. mansoni* life cycle are presented in Table 2.

### **Potential classes of epigenetic biomarkers applied to schistosomiasis diagnosis: Non-Coding RNAs, methylated DNA, and Histone post-translational modifications**

#### *Non-coding RNAs*

In the last decade, several research groups have performed studies in order to identify miRNAs in parasites of the genus *Schistosoma*, particularly *S. japonicum* and *S. mansoni*. A few currently known miRNA molecules were characterized based on data obtained by computational approaches associated with deep sequencing techniques (Cai *et al.*, 2013; De Souza *et al.*, 2011; Xue *et al.*, 2008). In the latest version of the genome and proteome of *S. mansoni*, it was established that the content of non-coding DNA sequences is 95.5 %, revealing the existence of several non-coding RNAs (ncRNAs) including transfer RNAs (tRNAs), small nucleolar RNAs (snoRNAs), miRNAs and small interfering RNAs (siRNA), and long non-coding RNAs (lncRNAs). The lncRNAs presented functional characteristics such as the presence of an epigenetic tag of transcriptional activation (H3K4me3), evolutionary conservation among other *Schistosoma* species, and differential expression at all stages of the parasite's life cycle (Maciel *et al.*, 2019; Vasconcelos *et al.*, 2017).

In the miRBase database, more than 3000 miRNAs have been catalogued, being 2661 of humans, 115 of *S. mansoni*, and 61 of *S. japonicum*. These miRNAs were identified and validated through computational resources associated with experimental methodologies such as northern blot, next generation sequencing, real-time PCR, and cloning among others (Kozomara & Griffiths-Jones, 2014).

In this context, since 2009, researchers in the Laboratory of Biochemistry and Molecular Biology of Universidade Federal de Ouro Preto, Brazil, are working on the characterization of miRNAs of *S. mansoni* transcriptome and the influence of infection on gene expression. Studies carried out this lab, using an integrated computational approach and systematic analysis of miRNAs, have identified 67 mature miRNAs and 42 precursors in *S. mansoni* (De Souza *et al.*, 2011; Pereira *et al.*, 2015). 211 new miRNAs had been characterized by cloning small sequences of RNAs obtained from adult *S. mansoni* (Simões *et al.*, 2011). Reports of 112 miRNAs using deep sequencing in adult worms (Marco *et al.*, 2013), and validation of 20 of the 42 characterized precursors and 2 of

Table 2. The epigenetic influence in different stages of the *S. mansoni* life cycle and its involvement in several mechanisms.

Life cycle stage	Developmental stage	Epigenetic Findings	Gene	Mechanisms	Reference
Mammal host	Mature egg	Epigenetic readers characterization	<i>SmMBD2/3</i> and <i>SmCBX</i>	Neoblasts proliferation and normal eggs production	(Geyer <i>et al.</i> , 2018)
	Schistosomula	miRNA enriched in RNA-seq analysis: oesophagus and tegumental cells derived.	sma-miR-277/4989	Downregulation in paired worms and upregulation in virgin worms – schistosomula to adult transition	Protasio <i>et al.</i> , 2017
	Adult worm	miRNAs parasite-derived in definitive host serum	miR-277, miR-3479-3p and bantam	Macrophage proliferation, TNF $\alpha$ increase and ovary development	Hoy <i>et al.</i> , 2014, Zhu <i>et al.</i> , 2016 Liu <i>et al.</i> , 2019
Free life	Miracidium	Mucin epigenetic polymorphisms	<i>SmPomucs</i>	Neutralizing snail immune system	(Fneich <i>et al.</i> , 2016; Perrin <i>et al.</i> , 2013)
	Cercaria	miRNA presenting high rates in a specific stage	miR-71	Stage specific functions	Mu <i>et al.</i> , 2019
Snail Host	Sporocyst	lncRNAs RNA-seq	Unique sets in sporocysts e.g SmLINC181757 SmLINC180219 SmLINC180220 SmLINC180221 SmLINC180222	Asexual reproduction, Kinome, Stage specific function	Kim <i>et al.</i> , 2020

the 211 miRNAs isolated in previous studies (Simões *et al.*, 2011). Eleven *Schistosoma* miRNAs were identified in the serum of *S. mansoni*-infected mice. The miRNAs Bantam, sma-miR-3473-3C, sma-miR-277, and sma-miR-3473-3C, of parasite origin, were considered as potential biomarkers for the diagnosis of schistosomiasis (Marco *et al.*, 2013).

The miRNAs are directly associated to the adequate development of ovaries in *Schistosoma* species. An example of the importance is related to the gametogenesis and egg production that is affected by the suppression of miR-31 and Bantam, conducting to the appearance of vacuoles in ovaries and tissue alterations in parasites (Zhu *et al.*, 2016). Bantam and Sma-miR-2c-3p are the two most expressed circulating miRNAs in the host blood. Thirty days after the start of oviposition, it is already possible to recover de-

tectable and expressive amounts of these molecules. As a result, methodologies are being developed to use circulating miRNAs as biomarkers, permitting disease diagnosis and clinical treatment as early as possible to avoid the occurrence of serious liver damage (Meningher *et al.*, 2017).

Among the ncRNAs, miRNAs play important regulatory roles in the differentiation and development of organisms. This has led to new findings of epigenetic mechanisms, although many mechanisms involved in these processes have not yet been fully elucidated (Geisler *et al.*, 2013; Holoch *et al.*, 2015). Due to its important biological role, circulating miRNAs were considered as potential biomarkers for the diagnostic of various diseases because specific miRNAs occur in body fluids such as plasma and serum at significant levels in a stable manner (Sandoval *et al.*, 2013).

*S. mansoni* is an extracellular parasite and thus, during its development, displacement, and installation in the definitive host, it releases several molecules that can be detected in the circulatory system of infected individuals (Skelly *et al.*, 2014). For this reason, it is possible to infer that molecules such as circulating RNAs in the serum or plasma of individuals with schistosomiasis could potentially be used as new epigenetic biomarkers (Meningher *et al.*, 2017; Mu *et al.*, 2019).

lncRNAs were detected in *S. mansoni*, and as they are part of regulatory mechanisms, many approaches have explored the change in the repertoire of these molecules in the transition of stages (Vasconcelos *et al.*, 2017), in different sexes and between susceptible and resistant strains (Oliveira *et al.*, 2018). Recently, changes in epigenetic marks have been associated with lncRNAs at different stages (Maciel *et al.*, 2019) and also a very distinct repertoire of lncRNAs has been detected in sporocysts that the authors associate with regulation of asexual reproduction (Kim *et al.*, 2020). Considering that lncRNAs have little sequence identity between species and that their functionality is more related to conformation than to pairing as in the case of miRNAs they could become good diagnostic biomarkers (Chen *et al.*, 2016).

#### Methylated DNA

Epigenetic modifications associated with DNA methylation, involving the incidence of lower (hypomethylation) or higher number of methyl groups (hypermethylation) have been frequently correlated to pathological conditions (Smith *et al.*, 2020). Therefore, the use of DNA methylation as a biomarker represents a great potential strategy for the diagnosis of helminth diseases, such as schistosomiasis.

There is a great deal of information about the enzymes involved in *Schistosoma spp.* histone alterations such as structural data, inhibitors, possible drugs, and others. However, there is still little knowledge about the mechanisms of DNA methylation in *Schistosoma*. Two different *S. mansoni* enzymes - DNA methyltransferase 2 (SmDNMT2) and methyl-CpG binding domain protein (SmMBD) - were demonstrated to be active and essential in DNA methylation as well as very conserved throughout the phylum (Geyer *et al.*, 2013).

There are data on the activity of DNA methyltransferase in the methylation of tRNA and the possible decrease or loss of its methylation properties (Raddatz *et al.*, 2013). However, research shows a possible methylation of the *Schistosoma* genome in CpG and CpA, and that the inhibition of this enzyme can maintain eggs in immature stages of development. This makes DNA methylation essential for the maintenance of the *Schistosoma sp.* life cycle (Geyer *et al.*, 2011; Geyer *et al.*, 2013; Raddatz *et al.*, 2013).

Previous studies have reported DNA methylation used as a biomarker for schistosome infection. In *S. haematobium* for example the promoter of *RASSF1A* and *TIMP3* genes is hypermethylated and can be used as a biomarker of *S. haematobium* infection (Zhong *et al.*, 2013). Disturbance in the global content of 5-mC and

in the expression of DNMTs and TETs (Ten eleven translocation) were detected in the liver of infected mice compared to uninfected maybe in the future some specific region or a set of expression profile could be used as schistosomiasis biomarker (Mota *et al.*, 2020).

By means of highly sensitive methods, the occurrence of DNA methylation was emphasized in the *S. mansoni* genome, which was inhibited in 5-azacytidine-treated adult worms, resulting in altered ovarian architecture and production of eggs with abnormal morphology (Geyer *et al.*, 2011). These authors also concluded that if DNA methylation occurs, specific enzymatic mechanisms are involved; however, these mechanisms are not yet known. Although there are specific biochemical processes for DNA methylation, genes that express enzymes like DNMTs and TETs, which are responsible for these mechanisms, were not found in *S. mansoni* (Geyer *et al.*, 2013). Thus, there is a group of researchers that reports the occurrence of DNA methylation and another that affirms the existence of some controversies regarding the methylation patterns (Fantappiè *et al.*, 2001; Raddatz *et al.*, 2013; Geyer *et al.*, 2013; Geyer *et al.*, 2018). This controversy demonstrates the need to obtain conclusive data to confirm or rule out the possibility of adopting *S. mansoni* DNA methylation as a biomarker.

#### Histone post-translational modifications

Post-translational modifications (PTMs) are highly dynamically regulated, and involve a set of specific enzymes for different chemical groups and amino acids, which can be: phosphate group, methyl, acetyl, proteins (like ubiquitin and SUMO), carbohydrates, ADP-ribosylation and response to nutrient availability, hormonal stimulation and cellular adaptation mechanisms under different physiological conditions (Wang *et al.*, 2014). Thus, changes in the dynamics of PTMs may have important pathological effects in response to the interaction of *Schistosoma* with its hosts. Histone modifying enzymes (HMEs), such as HATs, histone methyltransferases (HMTs), and HDACs have been described in *S. mansoni*. Knowledge about this family of enzymes opens perspectives for additional studies that will lead to a better identification of HMEs, their gene regulation, and the possible development of diagnostic and therapeutic strategies. HDAC enzymes, called Sirtuins (Sirt1, Sirt2, Sirt5, Sirt6, and Sirt7) are encoded by the *S. mansoni* genome, are NAD<sup>+</sup> dependent lysine deacetylases, and are expressed with distinct patterns of expression throughout the life cycle of the parasite. In the same work, the supplementation of sirtuin inhibitors in *S. mansoni* culture media culminated in schistosomula apoptosis. These inhibitors were able to induce unpairing of worms, alterations on ovary and testis architectures and reduction in oviposition (Lancelot *et al.*, 2013). Other example of targets linked to histone modifications is the HMT enzymes. In vitro assays containing GSK343 (a human EZH2 HMT inhibitor) led to reduction in schistosomula motility and adult worms displayed oviposition and morphology alterations. The authors suggested that the complex of repression Polycomb was affected by the HMT

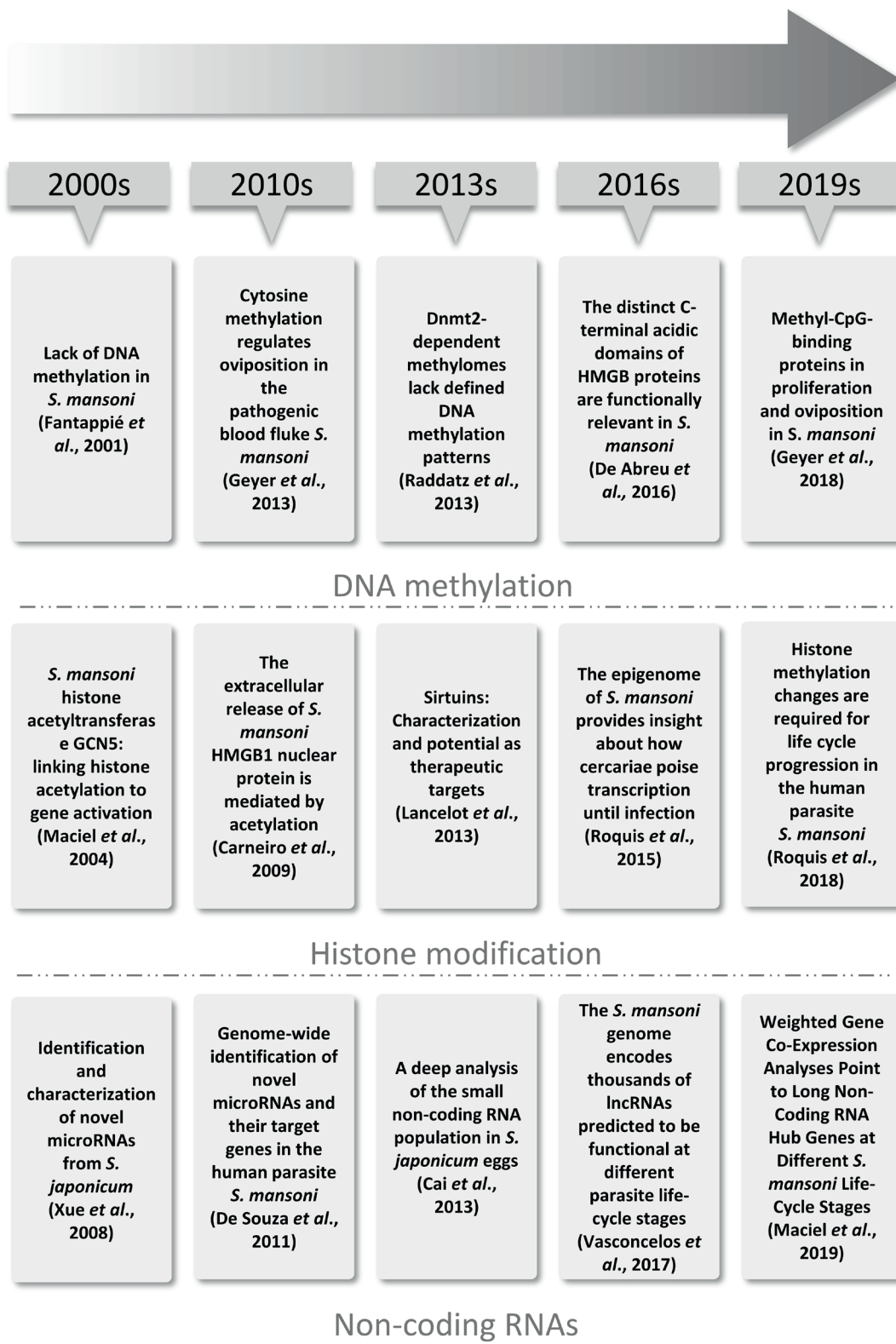


Fig. 2. Important advances in DNA methylation, histone modifications, and Non-coding RNAs related to *Schistosoma* in the last 20 years.



inhibition when they observed RNA metabolism genes and regulatory molecules gene expression with abnormal levels (Pereira *et al.*, 2018). Therefore, diverse HMEs were considered as potential therapeutic and diagnostic targets for schistosomiasis, because as in the case of sirtuin genes the sequence conservation was restricted to catalytic domains and these were expressed in all parasite life cycle probably due to the relevance of their functions. (Lancelot *et al.*, 2013; Pereira *et al.*, 2018).

Another example of therapeutic epigenetic targets was described in an essay that used *H. sapiens* erasers, writers and readers targets as queries to identify *S. mansoni* homologues. Inhibitors and miRNAs from these orthologues were supplemented in the cultures of adults and schistosomula causing morphological changes, and the inhibition of a specific HMT resulted in a decrease in the epigenetic mark H3K36me associated with a reduction in motility and in the quantity of eggs produced. These assays open perspectives of treatment during the schistosomula stage (Whatley *et al.*, 2019).

In the case of *S. mansoni*, depending on the susceptibility of the snail, miracidium expresses different configurations of a family of genes related to mucins. Among them, the *S. mansoni* polymorphic mucins (SmPoMucs) are proteins secreted by the apical gland that neutralize proteins of the snail defense system (Perrin *et al.*, 2013). During invasion, epigenetic regulation is best observed through a set of SmPoMucs because there is little change in the nucleotide sequence of the promoter region and the coding region. There are differences in the intensity of methylation and acetylation processes of histone H3K9 in the promoter region of these genes (Fneich *et al.*, 2016). Upon invading the definitive host, the male cercariae respond to chemical stimuli and the females to light stimuli, expressing the proteases necessary for penetration. These enzymes are stage-dependent, and it is probable that their transcription is activated by epigenetic mechanisms of DNA demethylation and/or acetylation of H3K9 (Roquis *et al.*, 2016).

Analysis of *S. mansoni* chromatin indicates that cercariae have low or no level of transcription, because several H3K27me3 repression tags, common in facultative heterochromatin, have been detected. Thus, the transcripts necessary for the survival of the parasite would be synthesized in the previous stages and would be sufficient until the infection of the definitive host, due to the short period of permanence at that stage (Picard *et al.*, 2016).

Adult *S. mansoni* displays regulatory mechanisms for reproduction, tegument formation, and steroid production, which have not been yet found in other stages of this parasite. There is a marked decrease in H3K27me3, due to the need for synthesis of new transcripts for final host installation (Roquis *et al.*, 2016).

Knowledge of the epigenetic signatures of *S. mansoni* and an understanding of the intriguing biological role of these molecules is fundamental for the clarification of regulatory processes, the immune response of the mammalian host, and the mechanisms of host and parasite interactions (Cabezas-Cruz *et al.*, 2014; Mota *et al.*, 2020). It is possible to assume that patterns involving DNA

methylation, histone modifications, and ncRNA can act as epigenetic biomarkers of schistosomiasis (Berdasco *et al.*, 2018). In this sense, it is necessary to gather collaborative efforts among the scientific community to solve the remaining challenges of the epigenetic biomarkers associated with schistosomiasis, so that new perspectives can be explored.

A timeline of important advances related to DNA methylation, histone modifications, and ncRNA observed for epigenetic biomarkers associated to schistosomiasis in the last 20 years is shown in Figure 2.

## Conclusions and future directions

Although there have been significant advances in the knowledge of genomes, it is still necessary to clarify the essential epigenetic mechanisms involved in the relationship between parasite and host that are responsible for specific diseases such as schistosomiasis; thus, contributing to improve the quality and efficacy of the diagnosis and current treatments (Dawson *et al.*, 2011).

During the *S. mansoni* life cycle, the parasite needs to adapt to different environmental conditions. With knowledge of the underlying molecular mechanisms it should be possible to mimic, alter, or block specific biochemical pathways in order to hinder or interrupt the transmission of schistosomiasis. In this scenario, the epigenetic contribution to disease is revealing especially relevant. There are already listed potential candidates to be used as biomarkers of schistosomiasis. However, the challenge is to achieve diagnosis based on epigenetic mechanisms as reproducible and accurate as possible. Moreover, the validation of the results will certainly open perspectives for the development of new biomarkers for both schistosomiasis and other helminthic diseases.

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## Conflict of Interest

The authors declare that they have no conflict of interests.

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