Signalling pathways in schistosomes: novel targets for control interventions against schistosomiasis

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Abstract

Over the last decade, there has been accumulating evidence showing that signalling pathways are involved in extensive biological and physiological processes in the human blood fluke schistosomes, playing essential roles in environmental sensing, host penetration, growth, development, maturation, embryogenesis, tissue self-renewal and survival. Due to the likelihood of resistance developing against praziquantel, the only drug currently available that is effective against all the human schistosome species, there is an urgent requirement for an alternative treatment, arguing for continuing research into novel or repurposed anti-schistosomal drugs. An increasing number of anti-cancer drugs are being developed which block abnormal signalling pathways, a feature that has stimulated interest in developing novel interventions against human schistosomiasis by targeting key cell signalling components. In this review, we discuss the functional characterization of signal transduction pathways in schistosomes and consider current challenges and future perspectives in this important area of research.

Introduction

Despite extensive efforts at control, the neglected disease of schistosomiasis afflicts more than 200 million individuals in 76 tropical and developing countries [1]. It is caused by three major clinically relevant species of blood flukes - *Schistosoma mansoni, S. japonicum* and *S. haematobium*. Infection with *S. mansoni* and *S. japonicum* results in hepatic and intestinal schistosomiasis, while *S. haematobium* infections result in urogenital schistosomiasis. The treatment of schistosomiasis is almost exclusively dependent on the long-term mass administration of the single available drug, praziquantel (PZQ), which has led to growing concerns about drug resistance [2]. No effective anti-schistosome vaccine is available.

Schistosomes are parasitic helminth worms which have a complex lifecycle involving an intermediate host (aquatic snail) and a mammalian definitive host, as well as free-living swimming stages (cercariae and miracidia) [3]. In contrast to other trematodes, schistosomes are dioecious and sexual development in the female worm is dependent on constant pairing with the male, through exquisite mechanisms that are not well understood. A molecular "dialogue" thus takes place not only between the parasite and environmental stimuli (signalling molecules from hosts, e.g. growth factors, neurotransmitters; light, and changes in osmolality and/or temperature during infection), but also between the male and female worms. Protein kinases (PKs) play key functional roles in signal transduction in controlling a broad range of biological processes such as cell growth, proliferation, metabolism, male-female interactions controlling oocyte and vitelline cell differentiation and fertility [4, 5], and are thus required to ensure schistosome survival and completion of their complex lifecycle. Understanding the process of signal transduction is an area that has stimulated particular interest by researchers aiming to develop novel interventions (i.e. drugs and vaccines) against

human schistosomiasis [6-9]. Based on their structure, PKs can be classified into eukaryotic protein kinases (ePKs) and atypical protein kinases (aPKs) [10]. The recent deciphering of the genomes of *S. mansoni* and *S. haematobium* identified over 250 ePKs [7, 11].

Functional characterization of signalling pathways in schistosomes

A broad range of methodologies have been employed to explore cell signalling in schistosomes, including *in silico* reconstruction of signalling pathways using transcriptome and genome data [12, 13]; functional prediction of pathway components by comparative genomics [5]; *in-situ* hybridization using 'smart' phospho-specific antibodies [14]; yeast two/three hybrid screening [15, 16]; and RNAi and chemical inhibition followed by observation of phenotype changes [17, 18]. Vaccine trials, using signalling components as candidate antigens followed by evaluation of their protection efficacy, have also been undertaken [19]. The application of these approaches has unravelled functional roles for particular signalling pathways/components in schistosome biology (Fig 1) as follows:

(1) A variety of PKs have been found to be specifically or predominantly expressed in gonads, and shown to be involved in development of the reproductive system, gametogenesis, and/or egg production [20]. These kinase include polo-like kinases (SmPlk1 and SmSak), Src kinase (SmTK3), Syk kinase (SmTK4), Src/Fyn kinase (SmTK5), Src/Abl kinase (SmTK6) and Abl-like PKs (SmAbl1 and SmAbl2) [4, 21], the receptor Ser/Thr (S/T) kinases (SmTβRI/II [22, 23]), receptor tyrosine kinases (venus kinase receptors (VKRs) [24, 25], insulin receptors (IRs) [19], schistosome epidermal growth factor receptor (SER) [16], fibroblast growth factor receptors (FGFRs) [26], mitogen-activated protein kinase (MAPK) [27], Protein kinase A (PKA) [28] and protein kinase C (PKC) [29].

(2) An insulin signalling pathway participates in regulating glucose metabolism in schistosomes, playing a pivotal role in worm growth, development and maturity [19, 30, 31].

(3) A fibroblast growth factor (FGF) signalling pathway plays a key role in the maintenance of adult stem cells and proliferation of germinal cells [32, 33].

(4) PKC and extracellular signal-regulated kinase (ERK) signalling potentially control the homeostasis of early schistosomula [34]; and modulation of the activities these two kinases affects schistosomule motility, phenotype, and reduces the survival rate of schistosomula.

(5) PKA signalling may mediate the response to host neurotransmitter stimuli by early stage schistosomula and adult worms, affecting parasite motility [14, 35]. Also, PKA signalling has been suggested to be required for regulation of cercarial viability and excretory processes [28, 35].

(6) Sensory protein kinase signalling (involving PKC, ERK and p38 MAPK) allows cercariae to respond to changes in light/temperature, and the presence of linoleic acid, and promotes host penetration [36]. In addition~~Also~~, p38 MAPK plays a role in regulating the ciliary-beat in ~~the~~ miracidia~~um~~ and in ~~during~~ sporocyst differentiation [37, 38].

(7) SmFes, a cytoplasmic tyrosine kinase in *S. mansoni*, may participate in penetration of the miracidium larval stage into the snail intermediate host, and help larval transformation after definitive host penetration [39].

Current challenges

Despite recent reports of key findings in the area of signal transduction in schistosomes, some challenges remain as are now described:

(1). The lack of an immortalized schistosome cell line means that molecular signalling experiments need to be carried out with whole intact schistosomes, worm lysates and/or primary cells, i.e., neoblast-like cells, or using other eukaryotic expression systems (i.e., yeast, *Xenopus* oocytes or mammalian cell-culture systems).

(2). ‘Smart’ phospho-specific antibodies have been used to detect the activation of pathway components after careful validation, but the approach has been limited to only a small number of schistosome ePKs [5]. The lack of commercial schistosome-specific antibodies against many of the components involved in cell signalling pathways represents another obstacle, although this could be potentially resolved by establishing a facility similar to the Malaria Research and Reference Reagent Resource Center (MR4) (https://www.beiresources.org/About/MR4.aspx) to provide a centralized resource for research reagents to the schistosomiasis scientific community.

(3). Though key new methodologies are available for dissecting the functional roles of signal pathway components in schistosomes, phenotypic changes observed or knock-down effects can only be maintained for a relatively short time and in particular developmental stages [17]. The CRISPR/Cas9 system has recently been adapted for genome editing in diverse organisms [40], including the human protozoan parasites *Toxoplasma gondii*, *Plasmodium falciparum*, *Trypanosoma cruzi* and *Leishmania* spp. [41]. Compared with RNA interference, CRISPR-Cas9 mediated editing has the potential to achieve long-term heritable gene manipulation in schistosomes. However, the application of CRISPR in schistosomes is still in its infancy [42].

(4). Inhibitors are powerful tools in characterizing the functional roles of ePKs~~,~~ but ~~yet~~ it is critical to interpret the data they generate ~~-~~due to specificity considerations, which is ~~not only~~ a problem not only in the context of distinguishing between parasite and host enzymes, but it is also the case that ~~as~~ some inhibitors ~~do not~~ have differing effects on ePK activity in organisms as phylogenetically diverse as~~t~~ flatworms and mammals. Also, unexpected ectopic effects may emerge due to inhibitors acting promiscuously depending on the concentration~~s~~ used or the concentration~~s~~ reached in a tissue.

(5). Developing vaccines targeting receptor kinases (e.g. as transmission-blocking vaccines) represents a novel path for developing control interventions [43]. Many anti-schistosome

vaccines have been tested using the laboratory mouse but it has been argued recently that this model in vaccination/challenge trials may be unsuitable for assessing vaccine efficacy [44]. Evaluating other animal models for vaccine studies or using larger or natural hosts of schistosome infection (e.g. bovines or non-human primates) in vaccine trials will thus be important but challenging due to the substantial increases in cost, the limited availability of reagents for analysing protective immune responses in these mammalian hosts, and ethical considerations.

(6). In regards to targeting cell signalling for anti-schistosome chemotherapy, in order to increase deleterious drug effects, studies of compounds targeting multiple kinases (given the redundancies in signalling pathways) [7] are warranted but this approach may increase the risk of non-specificity and side-effects.

(7). The most significant ~~greatest~~ challenge currently preventing the development of a new anti-schistosome drug is the lack of a prospect of profit, which has reduced the enthusiasm of pharmaceutical companies to develop new compounds against this neglected group of parasites. However, the constant and widespread use of low doses of praziquantel in global mass preventive chemotherapy programs for schistosomiasis in high prevalence endemic areas increases the threat of drug resistance developing [45], emphasising the need to continue research into developing new and effective compounds.

Future perspectives

Future in depth investigations of signalling pathways in vital *Schistosoma* cell types and/or tissues are clearly warranted. For example, a cohort of somatic stem cells, namely neoblast-like cells, have been isolated and identified in *S. mansoni*, and this group of cells can proliferate and differentiate into derivatives of multiple germ layers [32]. It has been shown that the *fgf* signalling pathway plays a key role in the maintenance of this cell population.

Yet, detailed information of the signalling cascade in this pathway remains elusive. Also recently, it has been found that mechanical injury results in both cell death and neoblast proliferation at wound sites in *S. mansoni* [46]. The scenario that schistosome neoblasts sense and transduce injury signals and in turn modulate their behaviour to repair damaged tissues is still unclear. In addition, neuromuscular signalling, induced by biogenic amines (i.e., acetylcholine, serotonin, dopamine and histamine), plays a pivotal role in mobility control and the schistosome pairing process [47-51]. Exploring signal transduction processes linking neuroactive receptors in the neuronal system of schistosomes will be of considerable interest. In this context, previous studies have confirmed the involvement of PKC and PKA in neurotransmitter receptor/G-protein mediated signal transduction in schistosomes [14, 36].

As key regulators mediating gene expression at the post-transcriptional level, miRNAs may serve as nodes in regulating signal pathways [52], adding a further layer of complexity to signal transduction. Although comprehensive miRNA expression has been profiled in various developmental stages or different sexes in schistosomes [53, 54], the process of cross-talk between miRNAs and cell signalling pathways in schistosomes is still unclear, but some temporal cues have emerged based on a limited number of studies. For instance, the expression profile of sja-miR-124-3p during different development stages [55] and the localization of sma-miR-124a-3p in the cephalic ganglia and in the nerve chords of adult worm [56], indicate its potential involvement in regulating signal transduction in the schistosome nervous system. Recently, Zhu *et al* [57] found that the male-biased expressed miRNAs, miR-8 and miR-3479 may regulate gene expression likely involved in the Wnt and TGF-β signalling pathways.

A number of PK inhibitors, including those approved by the US Food and Drug Administration (FDA) for cancer treatment, show efficacy as potential anti-schistosome drugs [18, 21]. In terms of disruption of egg laying and worm killing, ~~some~~ promising results have been obtained *in vitro* with tryphostin AG1024, which potentially targets IRs and VKRs [58], and Imatinib, which negatively affects the kinase activities of SmAbl1/2 and SmTK6 [59]. ~~from *in vitro* observation~~. However, experiments *in vivo* ~~experiments may~~ resulted in a ~~differ~~ markedly different outcome with Imatinib as the blood components alpha-1 acid glycoprotein and serum albumin reduced its killing efficacy [59]. Nevertheless, by screening ~~of~~ 114 anticancer compounds, Cowan and ~~colleagues~~ Keiser [60] identified two kinase inhibitors, trametinib and vandetanib, exhibiting ~~with~~ moderate anti-schistosomal properties, based on *in vivo* assays [60]. Inhibition of schistosome kinase activity by an RNAi-based approach caused severe anti-worm effects emphasising ~~indicating~~ the importance of MAPK signalling ~~in the~~ for parasite survival *in vivo* ~~survival~~ [27]. Recently, 40 protein kinases, considered essential for parasite survival, have been prioritised as druggable targets in *S. haematobium*, including fibroblast growth factor receptor and insulin receptors [7]. Owing to the functional and structural conservation of the catalytic domains between parasite and human ePK orthologs, new breakthrough points may be achieved by designing novel and effective inhibitors that can target sequences close to, but not in the ATP site, unusual accessory domains present in some PKs, or PKs specific to invertebrates such as members of the VKR family [61].

Furthermore, more sensitive molecular tools for schistosomiasis diagnosis have been developed recently [62, 63], which enables earlier detection of a schistosome infection. Developing effective drugs targeting and killing adolescent worms will not only prevent the development of chronic liver pathology (i.e. egg-induced granuloma formation and fibrosis),

the main clinical outcomes of hepatic schistosomiasis caused by the entrapped eggs in the liver tissues, but also help block transmission of the disease. In this regard~~ing~~, promising results have been obtained with ~~–~~two mTOR (mammalian target of rapamycin)-targeting inhibitors, temsirolimus and sirolimus, which killed newly transformed schistosomula within 24-48 hours [60].

Comment [d1]: Check as incorrect reference

Summary

- Important advances have been made in understanding facets of cell signalling in the human blood flukes and the functional characterization of several schistosome PKs using integrated approaches.
- Temporal cues highlight the potential for identifying new druggable and vaccine candidates critical for schistosome signalling transduction and parasite survival.

COMPETING INTERESTS

The authors declare that there are no competing interests associated with the manuscript.

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Figure Legend

Fig 1. Functional implications of signalling pathways/components throughout different developmental stages of schistosomes.