International Journal for Parasitology 46 (2016) 1–5

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Succinctus

Extracellular vesicles secreted by *Schistosoma mansoni* contain protein vaccine candidates

Javier Sotillo [a,*,1], Mark Pearson [a,*,1], Jeremy Potriquet [b], Luke Becker [a], Darren Pickering [a], Jason Mulvenna [b], Alex Loukas [a,*]

[a] Centre for Biodiscovery and Molecular Development of Therapeutics, Australian Institute of Tropical Health and Medicine, James Cook University, Cairns, QLD, Australia
[b] QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia

ARTICLE INFO

Article history:
Received 23 August 2015
Received in revised form 26 September 2015
Accepted 28 September 2015
Available online 13 October 2015

Keywords:
Schistosoma mansoni
Extracellular vesicles
Exosomes
Proteomics
Vaccine

ABSTRACT

Herein we show for the first time that *Schistosoma mansoni* adult worms secrete exosome-like extracellular vesicles ranging from 50 to 130 nm in size. Extracellular vesicles were collected from the excretory/secretory products of cultured adult flukes and purified by Optiprep density gradient, resulting in highly pure extracellular vesicle preparations as confirmed by transmission electron microscopy and Nanosight tracking analysis. Extracellular vesicle proteomic analysis showed numerous known vaccine candidates, potential virulence factors and molecules implicated in feeding. These findings provide new avenues for the exploration of host–schistosome interactions and offer a potential mechanism by which some vaccine antigens exert their protective efficacy.

Schistosomes infect >200 million people worldwide, mostly in tropical and developing regions. Schistosomiasis is responsible for the loss of 1.53 million disability-adjusted life years (DALYs) and up to 280,000 deaths annually in sub-Saharan Africa alone (Gryseels et al., 2006), and recent estimates indicate that the DALYs could be substantially higher if pathogenesis grading is revised to account for individuals who were traditionally classified as "asymptomatic" (King, 2015). Distinct organs and tissues are affected during human schistosomiasis due to the different tissue tropisms displayed by each species. The eggs of *Schistosoma mansoni* and *Schistosoma japonicum* are implicated in hepatosplenic inflammation and liver fibrosis when trapped in the portal vasculature and surrounding tissues (Gryseels et al., 2006), whereas the eggs from *Schistosoma haematobium* are trapped in the mesenteries surrounding the bladder and cause a range of sequelae including haematuria, anaemia, urosepsis, bladder cancer, and even increase the risk of acquiring HIV (Mayer and Fried, 2007).

The adult worm of *S. mansoni* inhabits the mesenteric plexus in the portal system where they live for up to 10 years, as a result of different immune-evasion strategies employed by the parasite (Collins et al., 2013). Key to the adult fluke's immunoevasive prowess is the tegumental bilayer, which allows the parasite to mask its own surface proteins and regenerate damaged tissues thanks to a population of adult stem cells that can differentiate into many cell types (Collins et al., 2013). Traditionally, the excretory/secretory (ES) and tegumental proteins from the different life stages of schistosomes have been analysed to study host–parasite interactions and to develop new therapeutic strategies against this parasite (Skelly and Wilson, 2006; Loukas et al., 2007; Sotillo et al., 2015). Interestingly, earlier publications aimed at characterising the ES products of adult schistosomes described the presence of proteins that were typically thought of as intracellular in location (Perez-Sanchez et al., 2006), and this was attributed to the presence of dying worms in culture. With the recent reports, however, of the secretion of extracellular vesicles (EVs) by different unicellular and multicellular parasites, including trematodes such as *Echinostoma caproni*, *Fasciola hepatica*, *Dicrocoelium dendriticum* (reviewed by Marcilla et al. (2014)), *S. japonicum* (Wang et al., 2015) and *Opisthorchis viverrini* (Chaiyadet et al., 2015), the cellular origins of many ES proteins have now been resolved.

EVs are membrane-bound vesicles that can be classified into two major types - exosomes and microvesicles - depending on their size and specific protein and RNA content. Parasite EVs can play roles in parasite–parasite and host–parasite communication (reviewed in Marcilla et al. (2014)), and have also been implicated

* Corresponding authors at: Centre for Biodiscovery and Molecular Development of Therapeutics, James Cook University, Cairns 4878, QLD, Australia.
E-mail addresses: javier.sotillo@jcu.edu.au (J. Sotillo), mark.pearson@jcu.edu.au (M. Pearson), alex.loukas@jcu.edu.au (A. Loukas).
[1] Both authors contributed equally to the manuscript.

http://dx.doi.org/10.1016/j.ijpara.2015.09.002
0020-7519/

in the pathogenesis of the carcinogenic liver fluke *O. viverrini* (Chaiyadet et al., 2015). Moreover, EVs from nematode parasites suppress T helper type 2 immune responses in mice and contain micro-RNAs (miRNAs) that suppress inflammatory cytokine production by epithelial cells (Buck et al., 2014), highlighting their immunoregulatory function and potential as therapeutic agents and diagnostic tools. Herein we provide, to our knowledge, the first evidence of EVs secreted by *S. mansoni* adult worms in vitro.

Schistosoma mansoni adult worms were obtained by perfusion from BALB/c mice at 8 weeks p.i. After culturing the worms (300 pairs) in serum-free Basch medium (Basch, 1981) at 37 °C, 5% CO_2 at a density of ~50 worm pairs in 4 mL of media for 7 days we collected the ES material and removed dead worms daily. EVs were purified using an Optiprep® discontinuous gradient (ODG) as described elsewhere (Mathivanan et al., 2010) with slight modification. Briefly, ES was subjected to differential centrifugation at 4 °C (500 *g*, 2,000 *g* and 4,000 *g* for 20 min each) to remove large parasite material such as eggs and tegumental debris. The supernatant was then concentrated using a 10 kDa spin concentrator (Merck Millipore, USA), ultracentrifuged for 45 min at 12,000 *g*, and the supernatant was subsequently ultracentrifuged for 3 h at 120,000 *g* at 4 °C using a MLS-50 rotor (Beckman Coulter, USA). The resultant pellet was resuspended in 70 µl of PBS and subjected to ODG separation. The ODG was prepared by diluting a 60% Iodixanol solution (Sigma Aldrich, USA) with 0.25 M sucrose in 10 mM Tris-HCl, pH 7.2 to make 40%, 20%, 10% and 5% iodixanol solutions and then 1.0 ml of these solutions was layered in decreasing density in an ultracentrifuge tube. The resuspended EVs were added to the top layer and ultracentrifuged at 120,000 *g* for 18 h at 4 °C. A control tube was similarly prepared using PBS instead of EVs to measure the density of the different fractions recovered from the gradient. A total of 12 fractions (330 µl each) were recovered from the ODG, each diluted with 8 ml of PBS containing 1 × EDTA-free protease inhibitor cocktail (Santa Cruz, USA), and concentrated using a 10 kDa spin concentrator to remove the excess Optiprep® solution. All fractions were kept at -80 °C until use. Fractions containing a 340 nm absorbance of 1.09-1.22 (fractions 7-10) were subjected to analysis by transmission electron microscopy (TEM) to detect the presence of EVs as described previously (Chaiyadet et al., 2015). Fractions 7-9 contained a clean preparation of EV-like vesicles, whereas fraction 10 contained both EVs and protein aggregates (Fig. 1). To confirm the presence and size of the vesicles from the fractions, an analysis of absolute size distribution and quantification of EVs from fractions 6-10 was performed by measuring the rate of Brownian motion and diffusion coefficient with a NanoSight LM10 system (NanoSight, Wiltshire, United Kingdom). Fractions 6 and 10 were also analysed to obtain more information about the purity of the preparations. EVs from fraction 7 had a size of 77.4 ± 34.8 nm, whereas the size of EVs in fractions 8 and 9 was 96.4 ± 34.8 nm and 97.9 ± 28.5 nm, respectively (Fig. 2). While it is generally unclear how to classify the different subpopulations of EVs (Webber and Clayton, 2013), particularly those of parasite origin (Marcilla et al., 2014), the size of EVs found in fractions 7–9 corresponds to exosome-like vesicles. The number of particles/ml varied from 8.362×10^9 in fraction 7 to 2.15×10^{10} in fraction 8 and 1.83×10^{10} in fraction 9 (Supplementary Fig. S1). Fractions 6 and 10 contained 2.46×10^9 and 3.55×10^9 particles/ml, respectively. The number of vesicles/µg of protein ratio (P/µg) has been proposed as a good approach to measure the purity of vesicle preparations (Webber and Clayton, 2013). The highest P/µg was found in fractions 8 and 9 (1.12×10^8 and 1.3×10^8 P/µg, respectively) (Supplementary Table S1), which agrees with the predictions from our TEM and Nanosight studies. Despite the ratios being lower than those proposed by Webber and Clayton (2013), the authors also suggested that EV preparations obtained from fluids other than cell culture or from different organisms could have different ratios as the protein content of the vesicles is not comparable between organisms (Webber and Clayton, 2013). Therefore, we propose that a ratio of 1×10^8 P/µg could represent high quality vesicular preparations from schistosomes, and perhaps other helminth parasites.

A total of 2 µg from each fraction was electrophoresed on an SDS-gel and silver-stained. While fractions 7–9 contained the majority of the proteins, there were significant differences with the other fractions, confirming the isolation of pure vesicles, and minimal contamination with other soluble proteins (Fig. 2). We believe this is the first time that ODG has been used to isolate and characterise the EVs from helminths, and it has proven to be a reliable method to obtain pure, clean vesicles from the ES products of *S. mansoni*. For the proteomic analysis of the vesicles, a total of 2 µg from fractions 7, 8 and 9 was combined and electrophoresed on a 15% SDS-gel. The gel was then cut into 12 slices and each slice was subjected to reduction, alkylation and trypsin digestion and analysed using the NanoSpray II of a QSTAR Elite instrument (Applied Biosystems, USA) as described previously (Sotillo et al., 2014). The acquired MS/MS spectra were searched against an in-house built database containing the *S. mansoni* predicted proteome from the publicly available genome (Berriman et al., 2009) and the proteins from the common Repository of Adventitious Proteins (cRAP, http://www.thegpm.org/crap/) appended with reversed decoy sequences using Mascot (Matrix Science; version 2.5) with a P value < 0.05, and against the same database using X!Tandem and MS-GF + search engines. Identification results were validated using PeptideShaker v0.41.1 (Vaudel et al., 2015); the False Discovery Rate was evaluated at Peptide Spectra matching-, peptide- and protein-levels and all levels were set at a maximum of 1%, and only proteins containing at least one unique peptide validated by PeptideShaker were considered as identified. The acquired MS/MS spectra were searched against the *Mus musculus* database from Uniprot to search for host proteins, but no significant results were obtained. We identified a total of 83 different *S. mansoni* proteins (Supplementary Table S2), including exosome markers such as the tetraspanins (TSP)s *Sm*-TSP-1, *Sm*-TSP-2 (the *S. mansoni* homologue of the typical human exosome marker CD63), *Sm*-TSP-4, TSP-18 and tetraspanin D76, Heat Shock protein (HSP)-70, enolase and elongation factor 1-alpha. Of the 83 identified proteins, 17 (20%) were homologous to proteins previously identified in EVs from *E. caproni*, 24 (29%), *F. hepatica*, 17 (20%) from *D. dendriticum* and 19 (23%) from *O. viverrini*, which implies a specific cargo in *S. mansoni* EVs compared with other trematodes, a difference probably attributed to schistosome-specific characteristics of parasitism such as sexual dimorphism, hematophagy and the final site of predilection in the host. While every effort was made to minimise the occurrence of eggs in the adult worm cultures (by removing media and eggs daily), we cannot discount the possibility of the presence of egg-derived EV proteins (if, indeed, they are secreted by eggs) in our preparation. A comparison of our proteome dataset with that of the egg secretome (Cass et al., 2007) revealed that, while there were proteins in common, the majority of these were molecules expressed in multiple life-cycle stages (e.g. annexin, ferritin, enolase), and the most abundant and unique egg-secreted proteins (e.g. Omega-1, IPSE/alpha-1 and Sm-p40) were not present in our dataset, indicating that the EV preparation was unlikely to be heavily contaminated with egg-derived EVs.

The most abundant protein in *S. mansoni* EVs, based on spectrum counting, is a saposin B domain-containing protein. Saposin-like proteins have been described from helminth parasites and are proposed to be haemolytic, playing a role in the nutrient acquisition process of haematophagous parasites by disrupting red blood cell (RBC) membranes to liberate haemoglobin (Don et al., 2008). A number of ferritin isoforms are also highly repre-

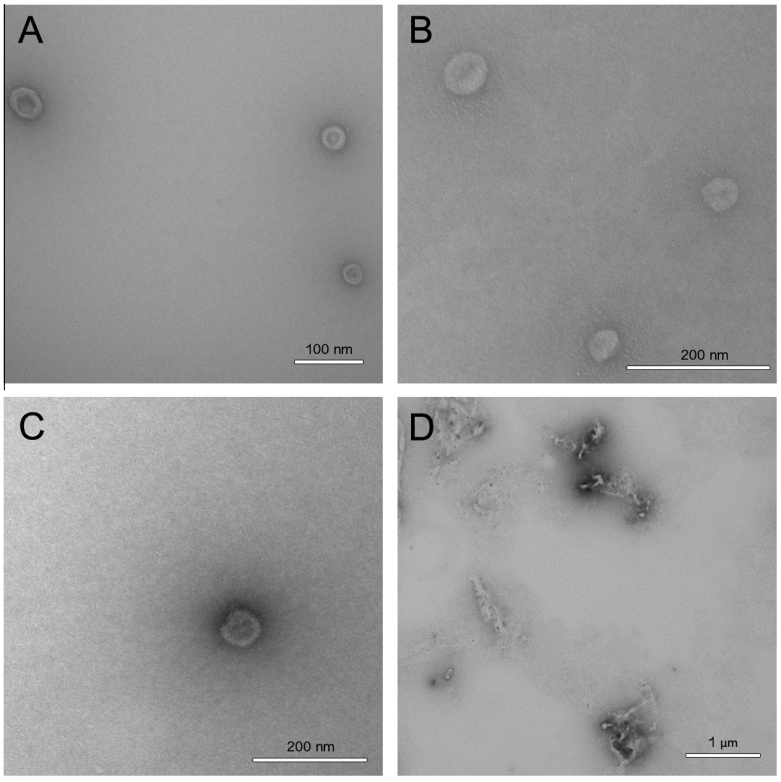

Fig. 1. Extracellular vesicles (EVs) secreted by *Schistosoma mansoni*. Transmission electron micrographs showing the presence of extracellular vesicles after Optiprep® gradient ultracentrifugation of *Schistosoma mansoni* excretory/secretory products. Fractions 7 (A), 8 (B), 9 (C) and 10 (D) were deposited onto a carbon-formvar coated grid, negatively stained in aqueous uranyl acetate and analysed on a JEM1011 transmission electron microscope.

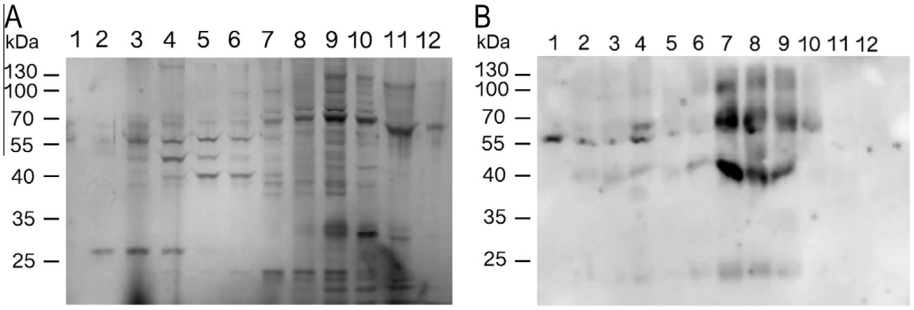

Fig. 2. Proteomic characterisation of the *Schistosoma mansoni* adult worm extracellular vesicles. The 12 fractions obtained from an Optiprep® density gradient were electrophoresed on a SDS-gel and silver-stained (A). The presence of *Sm*-TSP-2 was confirmed by western blot in the fractions containing extracellular vesicles (B). The lower band at ~23 kDa depicts monomeric *Sm*-TSP-2 and the higher bands at ~46 and ~69 kDa depict dimeric and trimeric forms of the protein. Moreover, *Sm*-TSP-2 was identified by tandem mass spectrometry in gel slices corresponding to both monomeric and dimeric forms, despite being reduced and alkylated.

sented. The presence of these haem-storage proteins in *S. mansoni* EVs, together with saposin-like proteins and other molecules involved in the feeding pathway (e.g.: aminopeptidases and metabolic enzymes), suggest a role for EVs in the nutrient acquisition process. An extension to this hypothesis is that *S. mansoni* EVs could be involved in cross-talk between paired male and female worms, perhaps in a nutrient transfer context, as there is evidence of EV-mediated communication between other parasites.

Interestingly, 26 (31%) of the identified proteins are homologues of previously described vaccine candidates or therapeutic agents (see Table 1). Some of these proteins (e.g. enolase, HSP-70 and Glutathione-S-transferase) are common to multiple life-cycle stages, including eggs (Cass et al., 2007) (Supplementary Table S2), implying that vaccines based on these proteins could simultaneously target different parasite stages. Moreover, four of the five most abundant proteins are known *S. mansoni* vaccine candidates or homologues of protective antigens from other flukes, including Saposin B domain-containing protein (Espino and Hillyer, 2003), *Sm*-TSP-2 (Tran et al., 2006), Sm29 (Cardoso et al., 2006) and cytoplasmic dynein light chain (Rezende et al., 2011). The fact that some of the proteins contained in *S. mansoni* EVs are efficacious in animal models as subunit vaccines (for example, Triose Phosphate Isomerase and Glyceraldehyde-6-Phosphate Dehydrogenase), previously presented a conundrum for vaccinologists as it was unclear how these antigens could be accessible to the immune system in a live fluke, but their presence in EVs implies a mechanism by which these molecules could egress from the fluke to be internalised by host cells, including antigen presenting cells. Given the important roles that EVs play in cell-to-cell communication and, presumably in the case of parasite EVs, the orchestration of parasitism, it is plausible that disruption of EV function and/or uptake by target cells through antibody-mediated neutralisation is a major mechanism by which these vaccines exert their efficacy. Indeed, TSPs have been described to play

Table 1
Homologues of vaccine candidates from different trematodes found in *Schistosoma mansoni* adult worm extracellular vesicles. Proteins are sorted by spectrum counting, which is related to the abundance of a protein in a particular sample. The proteins were also analysed for transmembrane domains.

Main accession no.	Description	Spectrum counting	Transmembrane domain	Homologues in other trematode EVs	Number of peptides
Smp_194910	Saposin B domain-containing protein	0.144736842	Yes	No	2
Smp_181530	TSP-2	0.112359551	Yes	Yes	4
Smp_072190	Sm29	0.086538462	No	No	2
Smp_040680	Cytoplasmic dynein light chain	0.073333333	No	Yes	4
Smp_130100	Saposin containing protein	0.066406250	No	No	3
Smp_045200	22.6 kda tegument antigen	0.033383459	No	No	13
Smp_059480	Thioredoxin peroxidase	0.031590414	No	Yes	6
Smp_116000	Annexin	0.022988506	No	Yes	2
Smp_077720	Annexin	0.019068946	No	Yes	16
Smp_158110.1	Thioredoxin peroxidase	0.016666667	No	Yes	1
Smp_074140	Annexin	0.016339869	No	Yes	3
Smp_068530	Syntenin	0.012755102	No	Yes	4
Smp_095520	Dynein light chain	0.011235955	No	Yes	4
Smp_086480	Antigen sm21.7	0.010869565	No	No	7
Smp_155310.1	TSP-1	0.009803922	Yes	Yes	3
Smp_102070	Glutathione S-transferase 26 kda (GST 26)	0.009174312	No	Yes	2
Smp_106930.1	Heat shock protein 70	0.008634560	No	Yes	15
Smp_030000	Leucine aminopeptidase (M17 family)	0.006830601	No	No	9
Smp_045560	Annexin	0.006734007	No	Yes	8
Smp_056970.1	Glyceraldehyde-3-phosphate dehydrogenase	0.002958580	No	Yes	3
Smp_074150.1	Annexin	0.002739726	No	Yes	1
Smp_024110	Enolase	0.002481390	No	Yes	7
Smp_045550	Annexin	0.002398082	No	Yes	2
Smp_104270	Ectonucleotide pyrophosphatase/ phosphodiesterase	0.002358491	Yes	No	4
Smp_009760	14-3-3 protein	0.001984127	No	Yes	2
Smp_157500	Calpain (C02 family)	0.001512859	No	No	4

a key role in the internalisation of *O. viverrini* EVs by human cholangiocytes (Chaiyadet et al., 2015), where their uptake by cells was blocked with anti-TSP antibodies, further supporting this hypothesis.

To confirm the presence of the *Sm*-TSP-2 vaccine antigen in *S. mansoni* EVs, we performed a western blot of the different fractions purified from the ODG with polyclonal anti-*Sm*-TSP-2 sera (Tran et al., 2006). A total of 2 μg of EVs from each fraction was separated on a 15% SDS-gel, transferred to nitrocellulose membranes, blocked overnight with PBS-Tween 0.05% (PBST) containing 5% skimmed milk and incubated with antisera to *Sm*-TSP-2 (1:500 in PBST) for 2.0 h at room temperature. Membranes were then probed with anti-rabbit IgG-horse radish peroxidae (HRP) (1:2000 in PBST) (Chemicon) for 1.0 h at room temperature and developed using the Amersham ECL Western Blotting Detection Kit. *Sm*-TSP-2 presence was confirmed in fractions 7–9; fractions 1–4 and fraction 6 were weakly positive, possibly due to contamination of these fractions with a small number of vesicles during ODG or sloughed tegument membrane that fractionated differently to EVs (Fig. 2B). We are now using anti-*Sm*-TSP-2 antibodies to attempt to purify *S. mansoni* EVs, in the same way that anti-CD63 antibodies are used to isolate human exosomes (Caby et al., 2005). The use of *Sm*-TSP-2 antibodies could also serve as a quality control reagent for *S. mansoni* EV isolation after ODG.

Herein we have isolated and characterised, to our knowledge for the first time, the EVs secreted by *S. mansoni* adult worms. These EVs contain numerous proteins known to be efficacious as anti-schistosome vaccines. Further investigation of *S. mansoni* EVs represents a unique avenue to explore *Schistosoma*–host relationships and holds significant potential for the design of novel therapeutics against this debilitating neglected tropical disease.

Acknowledgments

This work was supported by the program (1037304) grant from the National Health and Medical Research Council of Australia (NHMRC). AL is supported by a NHMRC principal research fellowship. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. All animal protocols were approved by the James Cook University Animal Ethics Committee (Animal ethics number A2004) and experiments were carried out in accordance with NHMRC guidelines. The following reagent was kindly provided by the National Institute of Allergy and Infectious Diseases (NIAID) Schistosomiasis Resource Center for distribution through BEI Resources, NIAID, National Institutes of Health (NIH): *Schistosoma mansoni*, Strain NMRI Exposed *Biomphalaria glabrata*, NR-21962.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpara.2015.09.002.

References

Basch, P.F., 1981. Cultivation of *Schistosoma mansoni* in vitro. I. Establishment of cultures from cercariae and development until pairing. J. Parasitol. 67, 179–185.

Berriman, M., Haas, B.J., LoVerde, P.T., Wilson, R.A., Dillon, G.P., Cerqueira, G.C., Mashiyama, S.T., Al-Lazikani, B., Andrade, L.F., Ashton, P.D., Aslett, M.A., Bartholomeu, D.C., Blandin, G., Caffrey, C.R., Coghlan, A., Coulson, R., Day, T.A., Delcher, A., DeMarco, R., Djikeng, A., Eyre, T., Gamble, J.A., Ghedin, E., Gu, Y., Hertz-Fowler, C., Hirai, H., Hirai, Y., Houston, R., Ivens, A., Johnston, D.A., Lacerda, D., Macedo, C.D., McVeigh, P., Ning, Z., Oliveira, G., Overington, J.P., Parkhill, J., Pertea, M., Pierce, R.J., Protasio, A.V., Quail, M.A., Rajandream, M.A., Rogers, J., Sajid, M., Salzberg, S.L., Stanke, M., Tivey, A.R., White, O., Williams, D. L., Wortman, J., Wu, W., Zamanian, M., Zerlotini, A., Fraser-Liggett, C.M., Barrell, B.G., El-Sayed, N.M., 2009. The genome of the blood fluke *Schistosoma mansoni*. Nature 460, 352–358.

Buck, A.H., Coakley, G., Simbari, F., McSorley, H.J., Quintana, J.F., Le Bihan, T., Kumar, S., Abreu-Goodger, C., Lear, M., Harcus, Y., Ceroni, A., Babayan, S.A., Blaxter, M., Ivens, A., Maizels, R.M., 2014. Exosomes secreted by nematode parasites transfer small RNAs to mammalian cells and modulate innate immunity. Nat. Commun. 5, 5488.

Caby, M.P., Lankar, D., Vincendeau-Scherrer, C., Raposo, G., Bonnerot, C., 2005. Exosomal-like vesicles are present in human blood plasma. Int. Immunol. 17, 879–887.

Cardoso, F.C., Pacifico, R.N., Mortara, R.A., Oliveira, S.C., 2006. Human antibody responses of patients living in endemic areas for schistosomiasis to the tegumental protein Sm29 identified through genomic studies. Clin. Exp. Immunol. 144, 382–391.

Cass, C.L., Johnson, J.R., Califf, L.L., Xu, T., Hernandez, H.J., Stadecker, M.J., Yates 3rd, J. R., Williams, D.L., 2007. Proteomic analysis of *Schistosoma mansoni* egg secretions. Mol. Biochem. Parasitol. 155, 84–93.

Chaiyadet, S., Sotillo, J., Smout, M., Cantacessi, C., Jones, M.K., Johnson, M.S., Turnbull, L., Whitchurch, C.B., Potriquet, J., Laohaviroj, M., Mulvenna, J., Brindley, P.J., Bethony, J.M., Laha, T., Sripa, B., Loukas, A., 2015. Carcinogenic liver fluke secretes extracellular vesicles that promote cholangiocytes to adopt a tumorigenic phenotype. J. Infect. Dis. http://dx.doi.org/10.1093/infdis/jiv291 (in press).

Collins 3rd, J.J., Wang, B., Lambrus, B.G., Tharp, M.E., Iyer, H., Newmark, P.A., 2013. Adult somatic stem cells in the human parasite *Schistosoma mansoni*. Nature 494, 476–479.

Don, T.A., Bethony, J.M., Loukas, A., 2008. Saposin-like proteins are expressed in the gastrodermis of *Schistosoma mansoni* and are immunogenic in natural infections. Int. J. Infect. Dis. 12, e39–e47.

Espino, A.M., Hillyer, G.V., 2003. Molecular cloning of a member of the *Fasciola hepatica* saposin-like protein family. J. Parasitol. 89, 545–552.

Gryseels, B., Polman, K., Clerinx, J., Kestens, L., 2006. Human schistosomiasis. Lancet 368, 1106–1118.

King, C.H., 2015. It's time to dispel the myth of "Asymptomatic" schistosomiasis. PLoS Negl. Trop. Dis. 9, e0003504.

Loukas, A., Tran, M., Pearson, M.S., 2007. Schistosome membrane proteins as vaccines. Int. J. Parasitol. 37, 257–263.

Marcilla, A., Martin-Jaular, L., Trelis, M., de Menezes-Neto, A., Osuna, A., Bernal, D., Fernandez-Becerra, C., Almeida, I.C., Del Portillo, H.A., 2014. Extracellular vesicles in parasitic diseases. J. Extracell. Vesicles 3, 25040.

Mathivanan, S., Ji, H., Simpson, R.J., 2010. Exosomes: extracellular organelles important in intercellular communication. J. Proteomics 73, 1907–1920.

Mayer, D.A., Fried, B., 2007. The role of helminth infections in carcinogenesis. Adv. Parasitol. 65, 239–296.

Perez-Sanchez, R., Ramajo-Hernandez, A., Ramajo-Martin, V., Oleaga, A., 2006. Proteomic analysis of the tegument and excretory–secretory products of adult *Schistosoma bovis* worms. Proteomics 6 (Suppl. 1), S226–S236.

Rezende, C.M., Silva, M.R., Santos, I.G., Silva, G.A., Gomes, D.A., Goes, A.M., 2011. Immunization with rP22 induces protective immunity against *Schistosoma mansoni*: effects on granuloma down-modulation and cytokine production. Immunol. Lett. 141, 123–133.

Skelly, P.J., Wilson, R.A., 2006. Making sense of the schistosome surface. Adv. Parasitol. 63, 185–284.

Sotillo, J., Sanchez-Flores, A., Cantacessi, C., Harcus, Y., Pickering, D., Bouchery, T., Camberis, M., Tang, S.C., Giacomin, P., Mulvenna, J., Mitreva, M., Berriman, M., LeGros, G., Maizels, R.M., Loukas, A., 2014. Secreted proteomes of different developmental stages of the gastrointestinal nematode *Nippostrongylus brasiliensis*. Mol. Cell. Proteomics 13, 2736–2751.

Sotillo, J., Pearson, M., Becker, L., Mulvenna, J., Loukas, A., 2015. A quantitative proteomic analysis of the tegumental proteins from *Schistosoma mansoni* schistosomula reveals novel potential therapeutic targets. Int. J. Parasitol. 45, 505–516.

Tran, M.H., Pearson, M.S., Bethony, J.M., Smyth, D.J., Jones, M.K., Duke, M., Don, T.A., McManus, D.P., Correa-Oliveira, R., Loukas, A., 2006. Tetraspanins on the surface of *Schistosoma mansoni* are protective antigens against schistosomiasis. Nat. Med. 12, 835–840.

Vaudel, M., Burkhart, J.M., Zahedi, R.P., Oveland, E., Berven, F.S., Sickmann, A., Martens, L., Barsnes, H., 2015. PeptideShaker enables reanalysis of MS-derived proteomics data sets. Nat. Biotechnol. 33, 22–24.

Wang, L., Li, Z., Shen, J., Liu, Z., Liang, J., Wu, X., Sun, X., Wu, Z., 2015. Exosome-like vesicles derived by *Schistosoma japonicum* adult worms mediates M1 type immune-activity of macrophage. Parasitol. Res. 114, 1865–1873.

Webber, J., Clayton, A., 2013. How pure are your vesicles? J. Extracell. Vesicles 2.