Characterisation of the *Schistosoma mansoni* Bromodomain containing protein 3 (SmBRD3)

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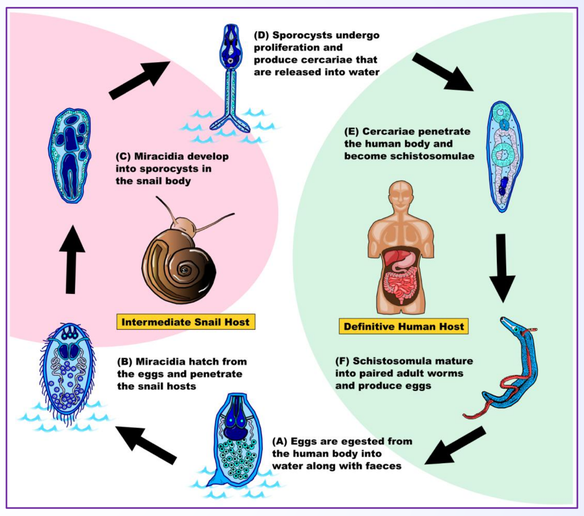
Background

- Schistosomiasis, a severely neglected tropical disease, ranks second only to malaria among the parasitic diseases in causing human suffering and death.
- The use of Praziquantel (PZQ), as the primary therapeutic agent for over a generation, raises concerns about potential resistance development.
- The *Schistosoma* life cycle contains different body plans in different stages. Epigenetic regulators are essential for regulating changes between stages.
- Bromodomain-containing protein 3 (BRD3) is a specific class of epigenetic reader that binds to acetylated lysine (KAc) residues on histones and is considered a potential therapeutic target for various diseases.

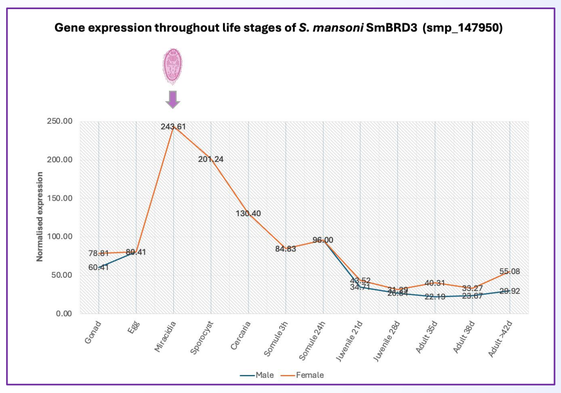


Life cycle of *Schistosoma mansoni* (Au et al., 2023)

- This study focuses on the essentiality of SmBRD3 by building on a previous study (1) that identified small-molecule inhibitors of SmBRD3.

Approach

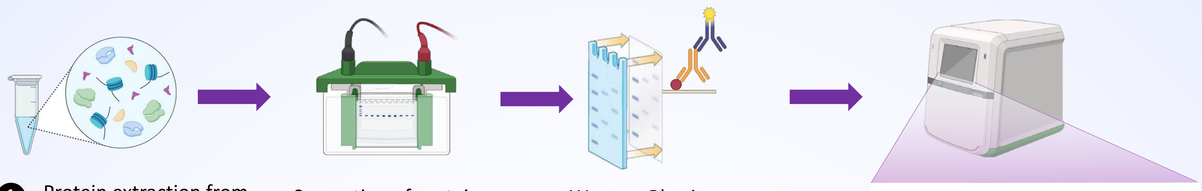
- *Smbrd3* is highly expressed in the *Schistosoma* miracidia.
- To gain a better understanding of SmBRD3 (Smp_147950), a previously generated antibody (anti-SmBRD3) raised against a recombinant form of SmBRD3 is being characterised.
- Due to the high expression of *Smbrd3* in miracidia, this parasite stage was used for initial immuno-assays (e.g., western blots).
- As previous studies demonstrated [2] that JQ1 (small molecule inhibitor) binds to BRD3 and affects the viability of schistosomula during 72 hr co-culture (at 10 µM), long-term JQ1 co-culture of schistosomula (at sub-lethal concentrations) is being used to assess the developmental importance of BRD3.



Normalised expression of this gene in different life stages, by mixed-sex infections, as analysed from published RNAseq studies (Lu et al., 2018)

Methods

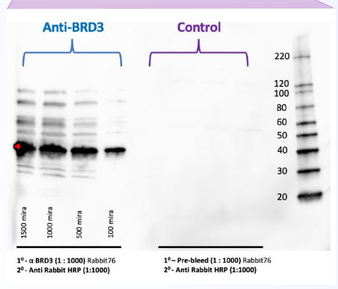
Characterisation of anti-SmBRD3



1. Protein extraction from the Miracidial samples using a protein extraction buffer containing Tris-HCl, NaCl, NP-40, and protease inhibitors.
2. Nuclear and cytoplasmic extraction from the adult worms using NE-PER extraction kit (Thermo scientific).

Separation of proteins according to their molecular masses by SDS PAGE.

Western Blotting – Probing with antibodies; Primary antibody – α BRD3 (1 : 1,000), Secondary antibody – Anti-Rabbit HRP (1:10,000).



Using a Chemiluminescence Imaging system, visualise and analyse immunoreactive proteins. .

The modulation of SmBRD3 during *in vitro* culture of the parasite

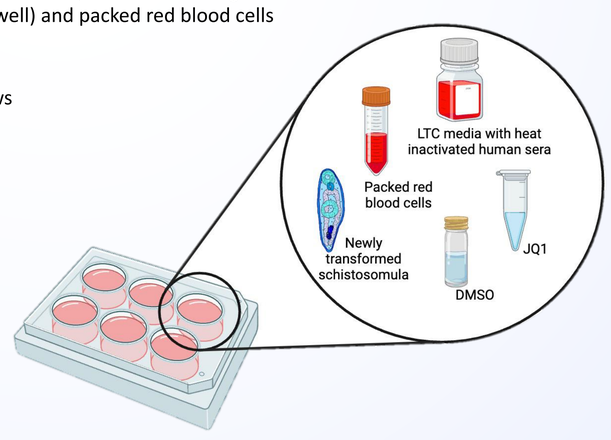
Under sterile conditions, newly transformed schistosomula were resuspended in 1X Long term culture (LTC) media with heat inactivated human sera (4ml/well) and packed red blood cells

Compound (JQ1) treatment was performed as follows
- DMSO (Positive control)
- 5 µM JQ1
- 2.5 µM JQ1
- 1.25 µM JQ1
- Media only

The culture plate was incubated at 37 ^{0}C, 5% CO_2

Media was changed weekly, and parasites were re-dosed as appropriate.

The development of schistosomula and changes in the phenotype was observed bi-weekly.

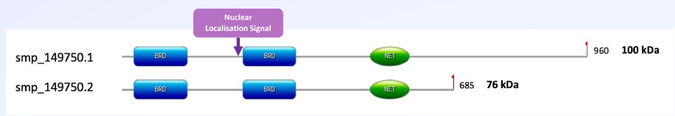


Objectives

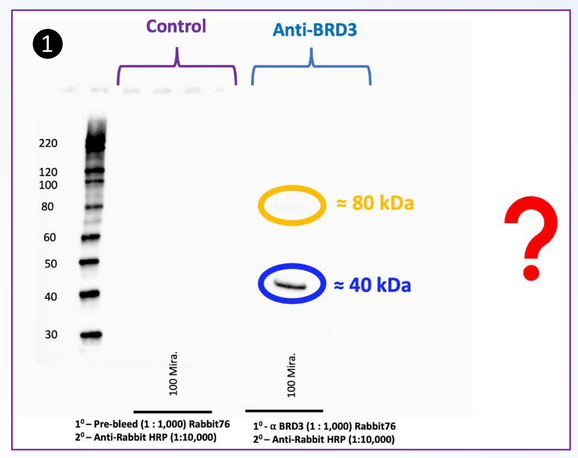
- Characterise SmBRD3's role in *S. mansoni* development.
- Characterise an anti-SmBRD3 antibody to further understand this epigenetic regulator.

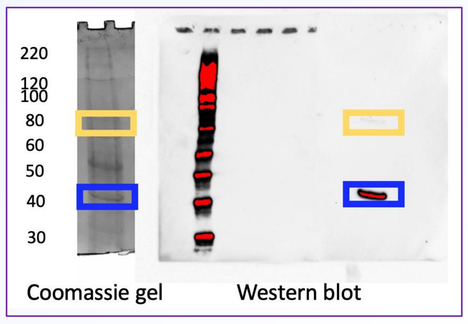
Results

Characterisation of anti-SmBRD3



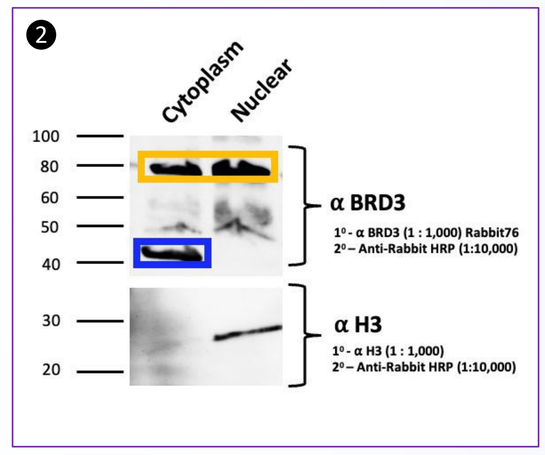
- SmBRD3 has two splice variants; SmBRD3_1 (smp_147950.1) and SmBRD3_2 (smp_147950.2) with predicted molecular masses of 100 kDa and 76 kDa, respectively.
- Western blots revealed Immunoreactive proteins at 40 and 80 kDa.
- While the 80 kDa is the predicted mass of SmBRD3_2, this protein is not as immunoreactive as the 40 kDa protein.
- To identify peptides in both immunoreactive bands, the proteins were subjected to mass spectrometry (MS-MS) for precise protein identification.





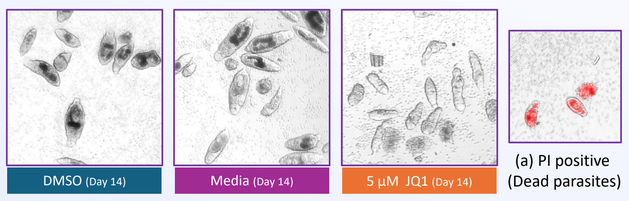
Repeat the procedure with miracidial protein extracts and SDS-PAGE, followed by Coomassie staining of solubilised proteins. Excise the interested protein bands in the Coomassie gel and subject them to Mass spectrometry to identify the proteins. Analyse the resulted data using specialised proteomic software to find the hits in our protein of interest (e.g., MASCOT).

- Proteomic results were unable to identify any peptides belonging to SmBRD3 protein (or other bromodomain-containing proteins).
- As SmBRD3 contains a nuclear localisation signal (NLS), nuclear vs cytoplasmic separation of miracidial extracts was next performed.
- While the 40 kDa protein seems enriched only in the cytoplasmic fraction, the 80 kDa protein is found in both nuclear and cytoplasmic fractions.
- Schistosome H3 is found predominantly in the nuclear fraction.

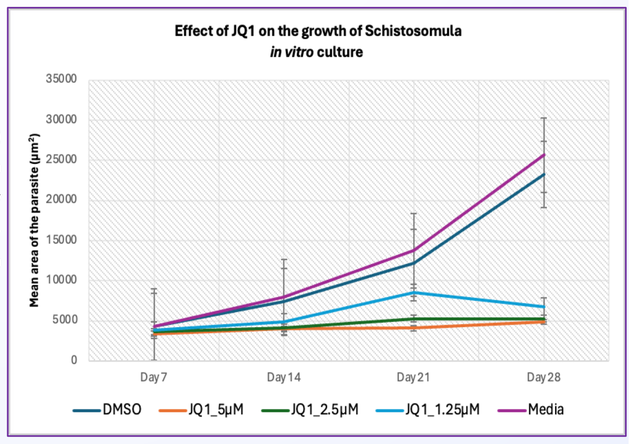


The modulation of SmBRD3 during in vitro culture of the parasite

- All sub-lethal concentrations of JQ1 (5-1.25 µM) inhibited schistosomula growth when compared to parasites cultured in either DMSO or Media-only conditions. While these parasites were not dead (PI-negative)[a], they failed to develop and were unable to feed normally (absence of haemozoin in guts).



(a) PI positive (Dead parasites)



Parasites treated with JQ1 exhibited significantly lower growth compared to the DMSO and Media only controls during the 28 days of culture.

- The above results suggest that SmBRD3 may be critical for the growth and development of Schistosomula.
- Other phenotypic features are currently being collected (e.g. stem cell numbers).

Future plans

- Further characterisation of anti-SmBRD3 will be performed using Immunoprecipitation - Immunoprecipitation of nuclear fractions, followed by MS-MS, could identify BRD3 and other interaction proteins.
- Continuation of the inhibition of SmBRD3 during *in vitro* culture of the parasite - Studying the effects of JQ1 and other small molecule inhibitors [1] of BRD3. This will be performed by monitoring the development of schistosomula over time to observe any changes in phenotype (growth rate, morphology, stem cell proliferation).
- RNAi - Investigate the functional Role of SmBRD3 using dsRNA – Suppressing the expression of the target gene to observe any phenotypic changes.

Conclusion

- The characterisation of an anti-SmBRD3 antibody may assist in our further understanding of SmBRD3 as an epigenetic regulator.
- The findings from this research could pave the way for the development of innovative strategies that target epigenetic regulators, offering a promising approach to combat parasitic infections.

References / Acknowledgments

(1) M. Schiedel, D. J. B. McArdle, G. Padalino, A. K. N. Chan, J. Forde-Thomas, M. McDonough, H. Whiteland, M. Beckmann, R. Cookson, K. F. Hoffmann and S. J. Conway (2023) Small Molecule Ligands of the BET-like Bromodomain, SmBRD3, Affect *Schistosoma mansoni* Survival, Oviposition, and Development, *Journal of Medicinal Chemistry*, 66(23), 15801-15822.
(2) K. C. L. Whatley, G. Padalino, H. Whiteland, K. K. Geyer, B. J. Hulme, I. W. Chalmers, J. Forde-Thomas, S. Ferla, A. Brancale and K. F. Hoffmann (2019) The repositioning of epigenetic probes/inhibitors identifies new anti-schistosomal lead compounds and chemotherapeutic targets, PLOS Neglected Tropical Diseases, 13(11), e0007693.

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