Biotechnology Advances 31 (2013) 1722–1737

Contents lists available at ScienceDirect

Biotechnology Advances

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

Research review paper

# *In vitro* cultivation of *Schistosoma japonicum*-parasites and cells

Qing Ye [a], Hui-Fen Dong [b], Christoph G. Grevelding [c], Min Hu [a,*]

[a] State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, Hubei, PR China
[b] Department of Parasitology, School of Basic Medical Science, Wuhan University, Wuhan 430071, Hubei, PR China
[c] Institute for Parasitology, Justus-Liebig-University, 35392 Giessen, Germany

ARTICLE INFO

*Article history:*
Received 5 May 2013
Received in revised form 6 September 2013
Accepted 8 September 2013
Available online 24 September 2013

*Keywords:*
*Schistosoma japonicum*
*In vitro* cultivation
Cell culture
Parasite culture

ABSTRACT

Schistosomiasis is a serious parasitic zoonosis caused by blood-dwelling flukes of the genus *Schistosoma*. Understanding functions of genes and proteins of this parasite is important for uncovering this pathogen's complex biology, which will provide valuable information to design new strategies for schistosomiasis control. Effective applications of molecular tools reported to investigate schistosome gene function, such as inhibitor studies and transgenesis, rely on the developments of *in vitro* cultivation system of this parasite and cells. Besides the *in vitro* culture studies dealing with *Schistosoma mansoni*, there are also numerous excellent studies about the *in vitro* cultivation of *Schistosoma japonicum*, which were performed by Chinese researchers and published in Chinese journals. Nearly every stage of the life-cycle of *S. japonicum*, including miracidia, mother sporocysts, cercariae, schistosomula, and egg-laying adult worms, was employed for developing *in vitro* cultivation methods, being accompanied by the introduction of several media and supplements that helped to improve culture conditions. It was not only possible to generate mother sporocysts from miracidia *in vitro*, but also to obtain adult worms from cercariae through *in vitro* cultivation. The main obstacles to complete the life cycle of *S. japonicum* in the lab are the transition from mother sporocysts to cercariae, and the production of fertilized and completely developed eggs by adult worms generated *in vitro*. With regard to cells from *S. japonicum*, besides established isolation protocols and morphological observations, media optimizations were conducted by using different chemical reagents, biological supplements and physical treatment. Among these, mutagens like N-methyl-N-nitro-N-nitrosoguanidine and the addition of extracellular matrix were found to be able to induce mitogenic activities. Although enzyme activities or the level of silver-stained nucleolar region associated protein in cultured cells indicated still suboptimal conditions, the achievements made point to the possibility of reaching the aim of establishing cell lines for *S. japonicum*. Both the improvements of the *in vitro* culture of larval and adult worms of *S. japonicum* as well as the access of cells of this parasite provide excellent advances for research on this important parasite in the future.



**Contents**



* Corresponding author at: State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, Hubei, PR China. Tel.: +86 27 87280032; fax: +86 27 87280408.
*E-mail addresses:* yeqing198173@gmail.com (Q. Ye), hfdong@whu.edu.cn (H.-F. Dong), Christoph.Grevelding@vetmed.uni-giessen.de (C.G. Grevelding), mhu@mail.hzau.edu.cn (M. Hu).

0734-9750/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.biotechadv.2013.09.003

## 1. Introduction

Schistosomiasis, a chronic and debilitating parasitic zoonosis, is ranked second after malaria in terms of worldwide public health significance, with more than 200 million people infected. Transmission of schistosomes, the pathogenic trematodes that cause schistosomiasis, is endemic over 70 tropical and subtropical countries (Hotez et al., 2008; Montresor et al., 2012), without well-established control measures. Besides humans, animals are also affected, which contributes to the enormous socioeconomic significance of schistosomiasis (Gryseels, 2012; Huang and Manderson, 2005; King, 2010; Zhou et al., 2012). The complex life-cycle of schistosomes, which includes sexual and asexual reproduction within vertebrate and invertebrate hosts, is a big challenge for the control of this parasitic disease. Paired male and female worms reside in the mesenteric veins of mammalian host, and the females produce a large quantity of eggs every day. Some of the eggs are discharged with the feces, while many become entrapped in the capillaries of the liver and other organs leading to serious pathological consequences within the definitive hosts.

Praziquantel (PZQ), a non-specific broad-spectrum anthelmintic drug, is used as main therapeutic treatment to control schistosomiasis. Due to its low cost and high efficiency, PZQ has been administered as the most frequently used drug to treat schistosomiasis in China, Egypt, Cambodia, Brazil and the other countries (Chen, 2006; da Rocha Pitta et al., 2013). However, since it is only effective in killing adult worms, PZQ is unable to prevent re-infection (Hang et al., 2001; Pica-Mattoccia and Cioli, 2004; Xiao et al., 1985). Moreover, reliance on and extensive use of PZQ also in mass treatment programs create the risk of reduced drug efficacy and emerging resistance, for which first evidence has been obtained (Botros et al., 2005; Doenhoff and Pica-Mattoccia, 2006; Doenhoff et al., 2009; Melman et al., 2009). Therefore, it is a highly desirable goal to develop new effective drugs or vaccines for schistosomiasis control (Beckmann et al., 2012a; Huang et al., 2012; Pitta et al., 2006; Sayed et al., 2008). However, this requires a full understanding of candidate gene/protein function.

Due to tremendous worldwide efforts genome sequencing projects produced the first draft genomes of *Schistosoma mansoni* (see Berriman et al., 2009; Protasio et al., 2012), *S. japonicum* (The *Schistosoma japonicum* Genome Sequencing and Functional Analysis Consortium, 2009), and *Schistosoma haematobium* (see Young et al., 2012). Although comprehensive knowledge about the gene repertoire of schistosomes now exists, the functions of most genes are still unknown. This impedes our understanding of this pathogen's complex biology, including its interaction with mammalian and mollusc hosts and the mechanisms involved in parasite development, and its reproduction.

Up to date molecular tools reported to investigate schistosome gene function and importance have mainly included pharmacologic inhibitor studies and transgenesis. Use of pharmacologic inhibitors of targeted proteins has proven to be a better approach to identifying the functions of enzyme- or receptor-encoding genes in schistosomes. These encoded proteins include thioredoxin glutathione reductase (TGR) (Kuntz et al., 2007; Song et al., 2012), protein kinase C (PKC) (Ludtmann et al., 2009), protein kinases related to the reproductive system (Beckmann et al., 2010; Knobloch et al., 2006; Leutner et al., 2011; Long et al., 2010), as well as insulin receptors (You et al., 2010). In a landmark series of reports on the transcriptome of *S. mansoni*, Verjovski-Almeida and colleagues noted the presence of transcripts encoding dicer and piwi/argonaute orthologues, indicating that an intact RNAi pathway may exist in schistosomes (Verjovski-Almeida et al., 2003; Krautz-Peterson and Skelly, 2008; C. Luo et al., 2010; R. Luo et al., 2010). At the same time, Skelly and colleagues established protocols for RNAi in *S. mansoni* using cultured schistosomula (Skelly et al., 2003). Subsequently, excellent studies were performed using RNAi for gene silencing to characterize various genes in cultured schistosomes (Beckmann et al., 2010, 2012b; Boyle et al., 2003; Cheng et al., 2005; Dinguirard and Yoshino, 2006; Kuntz et al., 2007; Osman et al., 2006). With respect to insertional transgenesis, biolistics (Davis et al., 1999; Grevelding, 2006), square-wave electroporation (Correnti et al., 2005; Krautz-Peterson et al., 2007; Morales et al., 2008; Ndegwa et al., 2007; Rinaldi et al., 2008), and soaking procedures (Boyle et al., 2003; Davis et al., 1999; Dinguirard and Yoshino, 2006; Freitas et al., 2007; Grevelding, 2006; Heyers et al., 2003; Rossi et al., 2003; Wippersteg et al., 2002a,b, 2003, 2005) were performed with mRNA, plasmid DNA, reporter-gene constructs or virions, and transient transformation was achieved. Furthermore, progress has also been made on stable transformation of schistosomes, since the genome integration of lentiviruses was demonstrated opening the possibility of gain-of-function and/or loss-of-function analyses in schistosomes (Mann et al., 2011; Rinaldi et al., 2012).

In spite of this progress, the lack of established cell lines, the inability to keep the entire life cycle of schistosome *in vitro*, and the limitations of existing culture techniques for juveniles or adults hamper the progress in the post-genomic era of schistosome research. Different *in vitro* culture systems not only for adult and larval schistosomes but also for isolated cells have been developed. These mainly focus on the *S. mansoni* and *S. japonicum*. Due to its zoonotic nature *S. japonicum* has the greatest impact on human and animal health of all schistosome species. Nevertheless, most of the known *in vitro* culture studies have dealt with *S. mansoni*, with only a few focusing on *S. japonicum* (see Mann et al., 2010; Quack et al., 2010). One of the reasons for this is that most studies about *in vitro* cultivation of *S. japonicum* were performed by Chinese researchers and published in Chinese journal rather than international ones leading to a communication deficit within the international community. This review summarizes relevant research achievements to close existing gaps and to exchange ideas about the improvement of schistosome *in vitro* cultivation. Within this article, we will 1) summarize approaches regarding *in vitro* culture of different developmental stages of *S. japonicum*, 2) review progress towards establishing cell culture systems, 3) discuss the application of established *in vitro* culture systems for functional studies and 4) propose future research directions.