# Chapter 6

## Discussion and conclusion

The discovery of *Schistosoma* spp. was made over a century ago. Since then multiple efforts have been made to decipher its life cycle and mechanisms required for successful infections. Although mammalian host bloodstream is often referred to as a "hostile environment", interactions and co-evolution between the parasite and the host have reached the point that the parasite is not only able to evade attack of the host immune responses, but also dependent on components of the immune responses and the bloodstream environment for their normal development. Furthermore, although the parasite lives at the expense of its host, it is important for *S. mansoni*, as a long-living parasite, that the host alive over a long period of infection. As a result, the balance between exploitation and reparation needs to be maintained.

The recent sequencing of the genome has moved *S. mansoni* research to a new level. In addition, since its development in 2008, RNA-seq technology has revolutionised large-scale studies of gene structures and expression. Its standardised methodologies yield vast amounts of data with minimal technical variabilities. Arguably, transcriptomic approach does have limitations. The approach cannot explain additional processes that occur after transcription of genes; it does not explain the extent to which a gene is translated. Furthermore, stability of protein products may also affect biological outcomes. However, transcriptomic approach has a huge benefit of being able to cover a broader scope compared to other high-throughput methods. It also requires minimal starting materials, meaning that samples which are challenging to obtain in sufficient quantities can be much more easily studied.

In this work, I incorporated high-throughput RNA-seq and available genomic information to deepen the understanding of *S. mansoni* interactions with its mammalian hosts. In chapter 3, I investigated changes in gene expression profiles of the parasite during its development in infected mice. One of the features that sets the dataset from the *in vivo* parasite apart from previous attempts is the inclusion of the lung stage as part of the infection timecourse, providing a novel resource for

investigating this early stage of infection. In chapter 4 and 5, *in vitro* experiments were set up where schistosomules were co-cultured with three types of human cells representing tissues that the parasite may encounter during its intra-mammalian infections. Transcriptomic profiles of the co-cultured schistosomules and co-cultured human cells were then explored.

## 6.1 Findings and interpretations

The findings in this thesis reflected key processes that could be important for *S. mansoni* infections and demonstrated that interactions with its host occurred in many biological aspects and through several changes in both the host and the parasite. One of the most fascinating aspects in the host-parasite interactions is how the parasite evade host defence mechanisms. This work agrees that multiple strategies for immune evasion are in place and it also predict a potential new player used by the parasite. The parasite also appeared to adapt to host environment in a way that it may benefit from metabolic resources available from the host. Finally, the interactions may not only support parasite survival, but also reduce pathology in the host.

### 6.1.1 Interactions with host defences

The interactions with host defences happen at multiple levels. Acquisition of host antigens alone cannot provide full protection from the host defences given that the parasite tegument is constantly shedded and that multiple parasite proteins and transporters are also present on tegument. Immunomodulatory strategies, therefore, could have essential roles. Majorities of the modulatory mechanisms discovered so far include parasite secreted and tegumental molecules that interact with immunological proteins produced by the host. In this thesis, additional potential player of the immunomodulation was revealed in chapter 3, where a predicted protein with no apparent sequence similarity but expressed at high level in lung stage, was found to have a clear structural similarity to the human CFH, an inhibitor of the complement cascade. Inhibition of complement cascade is likely to be important for *S. mansoni* survival, as demonstrated by multiple strategies of the parasite to inhibit host complement activation.

Interestingly, while the *in vivo* lung stage schistosomules and the adult stages up-regulated expression of a putative *S. mansoni* CFH-like gene (chapter 3), the exposure

of HUVEC to schistosomules *in vitro* led to down-regulation of host CFH-encoding genes (chapter 5). The down-regulation in HUVEC could be a secondary effect following the down-regulation of other inflammatory genes (leading to down-regulation of a regulator). Expression of *CFH* in endothelial cells during *S. mansoni in vivo* infections has not been investigated. However, if the down-regulation also occured *in vivo*, the up-regulation of the putative parasite *CFH-like* gene may not only protect the parasite but also supply CFH to the host, reducing host pathology. Further experiments on CFH level in endothelial cells of infected mice and on molecular activities of putative parasite CFH would be needed to support the proposition.

In addition, this thesis demonstrated that the modulation of the host immune responses may also happen via changes in host gene expression. In chapter 5, transcriptomes of the co-cultured HEPG2 cells, originated from hepatocytes, the main source of complement proteins, showed down-regulation of multiple components in complement and coagulation cascades. A previous study on livers from infected mice did not report reduction in gene expression of either complement or coagulation components (Wijayawardena *et al*., 2016). However, unlike the work in this thesis, the livers were obtained from mice infected with egg-laying adult worms which reside further away from the liver. The stages of the parasite, its distance from the liver, as well as the effects of eggs lodged in the liver might contribute to differences between studies. It would be interesting to investigate gene expression in the liver tissue during early liver stage. Alternatively, the gene expression of HEPG2 cells could be investigated when the cells were co-cultured with liver stage schistosomules obtained from infected mice.

Understanding how *S. mansoni* interacts with the host immune responses could lead to insights into the regulations of the immune system processes and how it can be modulated. The processes by which *S. mansoni*, and other parasites, modulate the host immune responses can be exploited for other practical purposes. For example, anti-coagulants from the parasite could lead to development of new treatment in blood-clotting disorders (Mebius *et al*., 2013); and immunomodulatory proteins from parasites could be used to treat immune-related diseases (Artis and Pearce, 2013; Croese *et al*., 2015).

### 6.1.2 Host reparation

Interactions with the host environment are required for parasite survival, and they may also help ensure survival of the host. For example, the immune responses against pathogens can damage host tissues; therefore, interference with the host immune responses may be beneficial to both the parasite and its host. HUVEC, originated from endothelial cells, displayed changes in gene expression related to the cell cycle and extracellular matrix remodelling, both of which are important for wound repair. Interestingly, these *in vitro* schistosomules were transcriptomically similar to *in vivo* lung schistosomules, which is the stage where schistosomules have been observed causing damages to endothelial cells in the lung (Crabtree and Wilson, 1986a). Changes in co-cultured HEPG2 also demonstrated that genes involved in liver tissue regeneration were up-regulated in the presence of schistosomules. Although damage is unlikely to have occurrd within the *in vitro* co-culture, the presence of the parasites may trigger the host cells to prepare for potential damage. A better understanding on this interaction and how the parasite might trigger the responses could also be applicable for other research on tissue repair.

### 6.1.3 Responses to environments

The work in this thesis showed that different mammalian tissues can induce distinct responses in the parasite *in vitro*; however, other host factors are required for development of the parasite as shown previously by many investigators (e.g. Clegg, 1965b; Shaker *et al*., 1998, 2011). Growth of schistosomules *in vitro* was limited to the early *in vivo* lung stage as demonstrated by the morphologies and transcriptomic profiles. Some of the changes in the *in vitro* schistosomules, however, suggested that the HEPG2 environment may have induced responses in schistosomules most similarly to the *in vivo* environment. Not only were the transcriptomes of the HEPG2 schistosomules most similar to the lung stage schistosomules *in vivo*, but all of the *MEGs* that were up-regulated in the lung stage schistosomules were also up-regulated in the HEPG2 schistosomules.

This up-regulation of specific *MEGs* in HEPG2 schistosomules and *in vivo* lung schistosomules provides striking evidence that *MEGs* are involved in host-parasite interactions. It supports previous work that characterised the roles of MEGs in host tissue invasion (DeMarco *et al*., 2010), immune evasion (Lopes *et al*., 2013), and

rapid evolutionary changes of certain MEGs that suggest diversifying selection from host interactions (Philippsen *et al*., 2015). In addition, this thesis work showed that the HEPG2 environment may provide certain signals that stimulate responses similar to the lung environment.

I have also demonstrated that *S. mansoni* may adapt to oxidative stress in their environment, consistent with existing knowledge on the importance of oxidation-reduction processes in schistosomes (Li *et al*., 2015). In chapter 3 and 4, genes involved in neutralisation of ROS and iron storage were up-regulated in the stages where oxidative stress could be a major challenge (in the *in vivo* lung, and in the HEPG2 co-culture). The lung may present an oxidative challenge as a result of high oxygen pressure, a by-product of the cellular respiration and of the immune responses against the parasite during its delayed migration in the lung. Liver hepatocytes, represented in this thesis with HEPG2, are the main site for detoxification and this leads to an environment with high oxidative stress. In addition, hepatocytes can respond to pathogens by producing ROS. During the *in vitro* co-culture, it is not clear whether the production of oxidative species were induced in HEPG2 cells. However, one of the GO terms enriched in the up-regulated genes was *responses to stress*. In addition, a gene encoding aldehyde dehydrogenase was up-regulated in *in vivo* schistosomules from the liver stages. As described in chapter 3, this gene may be involved in optimising aldehyde metabolism in host liver to obtain substrates for parasite energy requirement. However, aldehyde can also cause oxidative stress and is toxic; therefore, the roles of the parasite enzyme may include regulations of oxidative stress. Together, this highlights the importance of regulating oxidative stress in *S. mansoni* and demonstrates that the regulations may be responsive to their environment.

On the other hand, specific responses of co-cultured cell types show that host tissues may sense the presence of the schistosomules, although the mechanisms of detection are unclear. Some of the responses between the schistosomules and the host cells may be related. Co-cultured HUVEC (chapter 5) were up-regulated in multiple biological processes including *Notch signalling pathway*. The genes up-regulated included gene encoding Notch receptor (Notch4) and the effector transcription factors. In schistosomules co-cultured with HUVEC cells (chapter 4), down-regulated were

genes structurally similar to the immunoglobulin part of Notch ligands. Immunoglobulin domains could be involved in many other cell-cell interactions. The immunoglobulin part in the parasite protein might be able to bind to Notch receptors and its down-regulation in HUVEC schistosomules may prevent interference with host Notch signalling. However, the gene was up-regulated in the *in vivo* lung stage (chapter 3), when the parasite is frequently found in tight capillary spaces, and the expression was declined as the parasites developed into adult forms. Therefore, this suggested that the gene may require additional stimuli to promote its expression. Together with the down-regulation in HUVEC environment, it is possible that the gene could be involved in responding to and interacting with host endothelial cells and could be explored further.

In addition to Notch signalling, one of the key changes in co-cultured HUVEC (chapter 5) was genes involved in ECM organisation. Interactions with host ECM are not well described for *S. mansoni*, but they are thought to be important during invading stages that include penetration through host tissues (Yoshino *et al*., 2014). However, the roles of host ECM interactions during intramammalian stages are not established. The interactions appeared to occur at multiple levels. Schistosomules and adults of *S. mansoni* can degrade ECM as a result of their shed tegumental membrane (Keene *et al*., 1983). In this thesis, expression of genes related to ECM organisation (including collagens and protease enzymes involving in the remodelling) was affected in co-cultured HUVEC as well as HEPG2. The effects on host gene expression suggested that the remodelling of and interactions with host extracellular matrix could be important for *S. mansoni* infections. Some of the possible explanations are that ECM interactions may help the parasite adhere to host blood vessels, or prepare host tissues for reparation of damage as explained previously in chapter 5.

In contrast to co-cultured HUVEC, co-cultured HEPG2 (chapter 5) exhibited a considerably smaller number of genes affected by the co-cultured with schistosomules. A possible explanation is that the HEPG2 were present with schistosomules transcriptomically similar to the lung stage (chapter 4) instead of liver stages. Alternatively, HEPG2 do not grow in a monolayer, but instead grow in clusters and often form lumps. Cells at the centre of the lumps might not be affected

by the co-cultured schistosomules. As a result, the effects of schistosomules on cells on the periphery may be diluted.

## 6.2 Limitations

The possible dilution effect of HEPG2 can occur with any transcriptomic studies that include pooling of individual cells, or organisms. For example, *S. mansoni* samples from the *in vivo* timecourse were used as whole worms which contained mixtures of tissue types. If the changes over the timecourse only happened in certain tissues, they may have been masked out by changes in tissues which were more abundant or fluctuated over time. Such an effect has been described as Simpson's paradox, which explains a common problem in identifying individual pattern when the data is a pool of multiple individual set of data (Simpson, 1951; Trapnell, 2015). The trend of changes might present when each dataset is considered separately, but disappear when all datasets are pooled. For example, given a tissue of mixed cell types, if a gene was up-regulated in one cell type but down-regulated in another cell type, the changes may not be detectable when RNA came from the whole tissue. Likewise, if one cell type is more abundant than others, its effects may represent the whole tissue even though other cell types might respond differently.

Current transcriptomic approaches cannot avoid the issues of pooled cell types, but modifications and technological development could improve future attempts. For example, in the case of HEPG2, a different type of hepatocyte-derived cells could be used that grow as a monolayer; alternatively, a single-cell transcriptomic approach could separate affected and un-affected cells. For *S. mansoni*, techniques have been developed for isolation of certain tissues such as reproductive organs (Lu *et al*., 2016) and gastrodermis (Gobert *et al*., 2009; Nawaratna *et al*., 2014). However, isolation of other tissues, such as cells of the nervous system, is still limited. Potential next steps to solve these issues and to obtain increasingly informative data could involve obtaining transcriptomes from single worms, or single cells from dissociated worms. Current constraints for this endeavour are laboratory procedures for dissociating each stage of the parasite into single cells effectively and optimally, and the handling and analysing massive amount of information that this approach will lead to.

Validating host responses *in vivo*, if using a transcriptomic approach, is also limited by the issue of pooling cell types. Extracting RNA from a mass of tissue types would not be ideal for studying the effects on the host cells that are in contact with the parasite. A proteomic study has shown that surface proteins could be isolated from other cellular proteins using biotin tagging followed by isolation of the proteins from dissociated cells (Torre-escudero *et al*., 2014). It could be appealing to experiment whether surface cells could be isolated from dissociated organs using a similar approach.

## 6.3 Concluding remarks

Transcriptomic data from this thesis provide further understanding of biological processes involved in intramammalian stages of *S. mansoni* infections, particularly on the aspects of immune evasion, and the responses between the parasite and its host. The datasets produced, and the genes and biological processes identified will provide a rich new resource for data mining and functional characterisation by the research community. Understanding the functions of parasite genes could lead to identifying targets for intervention and provide better overall insights into how the parasite functions within its host.

Finally, this work reflects the importance of incorporating multiple approaches into a research problem. The value of using multiple bioinformatic tools for functional predictions are demonstrated, but the idea can be extended to the incorporation between the high-throughput approaches and the functional characterisation of an individual gene or pathway. To study host-parasite molecular interactions, for *S. mansoni* and mammalian hosts, each organism possesses over 10,000 genes responsible for multiple interlinking biological processes. High-throughput approaches have provided a broad perspective, identifying putative processes that may be interconnected, and determining genes or processes that may have key roles. The putative new roles and functions from the current study will need to be validated, but the dataset has provided new starting points in the exploration of *S. mansoni* biology and in the search for vulnerabilities that may ultimately be exploited to combat and control schistosomiasis.