Chapter 5

Transcriptomes of cell lines exposed to schistosomules *in vitro*

5.1 Introduction

5.1.1 Overview

From chapter 4, I conducted a co-culture experiment between human-derived cells and *S. mansoni* schistosomules and studied how the parasites responded to the presence of the human cells. The three cell types used were HUVEC (human umbilical vein endothelial cells), HEPG2 (hepatocyte cancer cells), and GripTite (modified human embryonic kidney (HEK) cells). The overarching goal was to better understand the effect of the encounter in both the parasites and the host cells, and how this may contribute to the overall infection outcome. This chapter, therefore, covers the effect of the co-culture on the host cells. I investigated transcriptomic profiles of the co-cultured human cells, examined processes that were affected, and interpreted their relevance in the infections. This information, although derived from an *in vitro* system (with human cells forming an underlying layer and schistosomules placed on top), provides insights into host responses that the parasites encounter during an *in vivo* infection.

5.1.2 Host responses in *S. mansoni* infections

Systemic host responses have been explained largely in terms of changes in host immunological profiles during *S. mansoni* infections. The infections with *S. mansoni* appeared to suppress the inflammatory responses that would otherwise clear the infection (Pearce and MacDonald, 2002; Pearce *et al*., 2004). It has been proposed that the parasites achieve this by camouflaging themselves with host molecules (Goldring *et al*., 1976; Sell and Dean, 1972; Smithers *et al*., 1969). Furthermore, the parasites secrete molecules that modulate host immune responses, reducing inflammation (Ranasinghe *et al*., 2015a; Rao and Ramaswamy, 2000). Additionally, the parasites inhibit the attack by complement components by expressing genes that

prevent complement cascade activation, or degrade certain complement components (Da'dara *et al*., 2016a; Marikovsky *et al*., 1986; Schroeder *et al*., 2009; Skelly, 2004). The parasites clearly interact with their environment promoting the infection success. Moreover, the parasites are in close proximity to host tissues such as endothelial lining of blood vessels, and many cell types in the liver. These tissues are involved in pathogen infections; for examples, endothelial cells can express multiple cytokines and leukocyte adhesion molecules; and liver parenchymal tissue (hepatocytes) is the main factory of many molecules in complement and coagulation cascades. The proximity between *S. mansoni* and these tissues might allow interactions and modulation of such relevant processes.

5.1.3 Host tissue responding to *S. mansoni*

Endothelial cells are affected by *Schistosoma* infection. Treated with schistosomule ES, endothelial cells were polarised toward an anti-inflammatory profile that included changes to surface protein expression and second messenger signalling pathways (Angeli *et al*., 2001; Trottein *et al*., 1999a, 1999b). Furthermore, endothelial cells in the lung may be damaged during the lung phase migration due to the limited capillary spaces. However, lungs of infected rodents only show inflammation and wound healing processes after the parasites have left the tissue (Burke *et al*., 2011; Torre-escudero *et al*., 2014).

As for the liver tissues, information on the liver responses to schistosome infections have overwhelmingly focused on interactions with eggs and the formation of granuloma (e.g. Colley et al., 2014; Hams et al., 2013), with scarce information on how the liver is affected in early stages of an infection. Blood capillaries inside the liver are sinusoidal, allowing transport of large molecules between the blood vessels and the hepatocytes. The hepatocytes lying adjacent to blood vessels, therefore, might be affected by the migrating and developing parasites.

5.1.4 Aims and approaches

The aim of this chapter is to gain further insight into how cells of mammalian host respond to *S. mansoni*, in order to better understand the altered biological processes which may be important for the infections. Using co-culture set up between schistosomules and human-derived cells, I demonstrated in chapter 4 that

schistosomules changed their gene expression in response to different human cell types. In this chapter, I investigated transcriptional changes in the co-cultured human cells to find out how they were affected by the schistosomules.

5.1.5 Outline

In chapter 4, I described the co-cultured experiment where three types of *in vitro*-adapted human cells were co-cultured with schistosomules. Both the human cell and the schistosomule samples were processed for RNA-seq and in chapter 4 the transcriptomes of the schistosomules were investigated. In this chapter, the transcriptomes of the human cells were studied. Two types of the cells, although originating from tissues relevant to *S. mansoni* infections (endothelial cells and hepatocytes), have been adapted to an *in vitro* environment and may have changed in their physiology. Therefore, in the first part, I assessed HUVEC in comparison to *in vivo* endothelial cells, and HEPG2 in comparison to *in vivo* hepatocytes. In the second part, I explored variation in transcriptomic profiles of all the human cell samples and investigated each cell type individually using differential expression and functional enrichment analyses. Finally, responses of genes in two pathways, selected from pathway enrichment analyses and their relevance to *S. mansoni* infections, were compared between cell types. Overall, this chapter confirms some of the known key host responses but also augments our knowledge with additional details not previously reported.

5.2 Methods

The human cells used for producing transcriptome dataset for this chapter were obtained as described in chapter 4. The experimental design is summarised here.

Three types of cells derived from human tissue were co-cultured with mechanically transformed schistosomules over a period of time, in Basch media. The cell types were HUVEC (human umbilical vein endothelial cells), HEPG2 (hepatocyte cancer cells), and GripTite (modified human embryonic kidney cells, HEK293). Human erythrocytes were added to the media from day three onward. Every 3-4 days, the schistosomules were transferred to new plates of cells until day 6, 10, and 17 after the start of the experiment (i.e. age of schistosomules after the transformation) when the schistosomules and cells were collected for downstream processing for transcriptome

analysis (co-cultured groups). At these time points, human cells were four days old after being plated and were co-cultured with schistosomules for three days regardless of the schistosomule ages; therefore, control cells were four days old cultured without schistosomulesfor three days in Basch media supplemented with human erythrocytes (worm-free groups).

The methods for downstream processing including RNA extraction, sequencing library preparation, and bioinformatic analysis are as described in chapter 2.

5.3 Results

5.3.1 RNA quantity and quality

The yield of extracted RNA ranged from 17-750 ng/µl, with the majority having RIN number of 10 (the lowest was 9.10) indicating non-degraded RNA. The purity of RNA, measured by NanoDrop spectrometre 1.0, however, were compromised in various samples. The 260/230 ratio, but not 260/280 ratio, was low in RNA extracted from HUVEC and HEPG2 cells. (Figure 5.1). The low 260/230 ratio can be an indicator of contamination with carbohydrate, or residual phenol or guanidine from the RNA extraction (Thermo Scientific, 2011). Carbohydrate carryover is most likely. Hepatocytes, represented by HEPG2, store glycogen. And endothelial cells, represented by HUVEC, are coated with glycocalyx (Reitsma *et al*., 2007). The samples were proceeded into RNA library preparation without further clean up because mRNA would be pulled down with oligo-dT beads, removing the RNA from the contaminants. One sample failed at library preparation step but this was not related to the low 260/230 ratio.

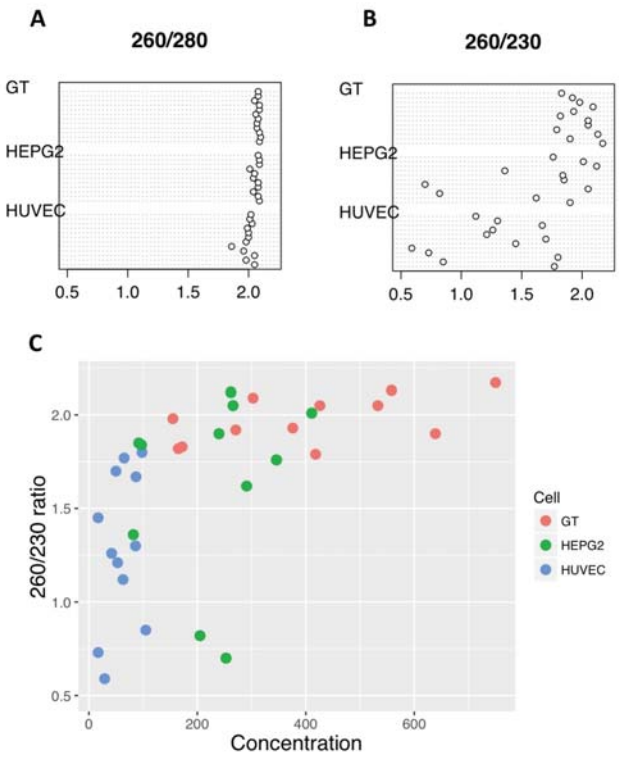

Figure 5.1 260/230 ratio of RNA extracted from human cells and its relationship with the RNA concentration

Purity of RNA samples from three cell types as indicated by 260/280 ratio (A) and 260/230 ratio (B) measured from NanoDrop Spectrophotometer. C) Relationship between RNA concentration and the 260/230 ratio.

5.3.2 HUVEC and endothelial cell surface markers

Although HUVEC were derived from endothelial cells, they have been adapted to an *in vitro* culture environment and may only retain some aspects of true endothelial cells. The full extent of the differences could not be revealed without the original endothelial cells. Instead, HUVEC were assessed for their likeness to *in vivo* endothelial cells based on expression of known endothelial cell surface markers obtained from (Durr *et al*., 2004), containing 70 markers from humans, rats, and mice. Uniprot identifiers, if provided, were used to retrieve the identifiers of their human orthologues from the NCBI database. Where the identifiers were not provided, product names were used to search for human orthologues. The original list of genes from (Durr *et al*., 2004) and their matched human orthologues are provided in Appendix G. Defining expressed genes as having an FPKM greater than 0 in all replicates in worm-free HUVEC, 64 genes out of 70 genes (91.4%) were expressed in

worm-free HUVEC (Figure 5.2). Although, it is not possible to determine the full extent of differences between HUVEC and true endothelial cells nor the downstream processes once the surface interactions have been initiated, the presence of most of the cell surface markers confirms that the *in vitro* cells are capable of many of the environmental interactions that their *in vivo* counterparts perform.

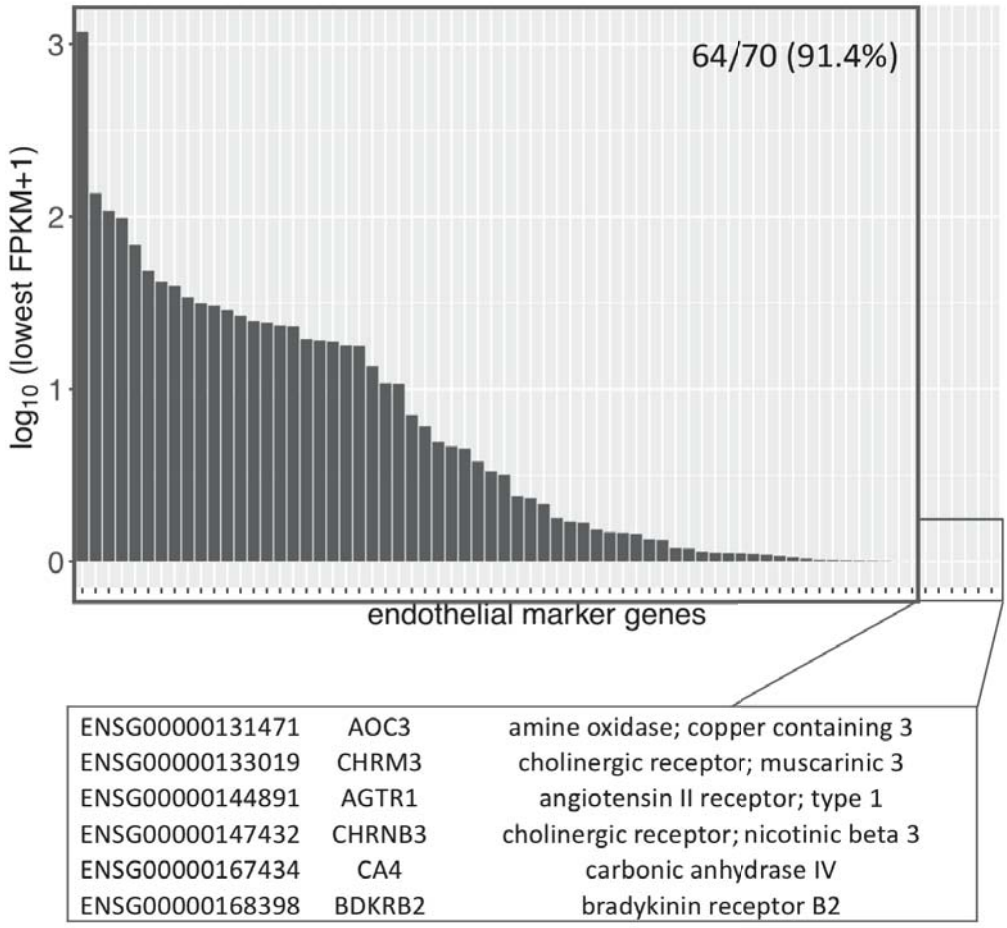

Figure 5.2 Expression levels of endothelial cell surface markers in worm-free HUVEC
Endothelial surface marker genes from Durr *et al.* (2004) and their expression levels in worm-free HUVEC. The expression levels shown on y-axis are the lowest FPKM amongst all three replicates of worm-free HUVEC. Each tick on x-axis represent one gene. Six genes at the right hand side were not expressed in any replicate of worm-free HUVEC. Two of the genes were expressed at a lower level that their levels are not visible on the plot. 91.4% of the endothelial cell markers were expressed in worm-free HUVEC (at least one replicate with FPKM > 0).

5.3.3 HEPG2 and liver hepatocytes

Similarly, HEPG2 were derived from hepatocyte cancer cells and would also have adapted to *in vitro* environment. The comparison between HEPG2 and liver hepatocytes has been done previously using RNA-seq expression comparing HEPG2 with liver tissue (Tyakht *et al*., 2014). The study shows that 50 genes were up-regulated in HEPG2 cells while 608 genes were up-regulated in the liver tissue. The 50 genes were enriched in cell cycle and cell division, reflecting the carcinoma nature of the cell line (Tyakht *et al*., 2014) and positive selection for fast-growing cells *in vitro*. The 608 genes up-regulated in liver tissue, are enriched in processes of liver function such as metabolism and innate immune responses (Tyakht *et al*., 2014). Incoporating the data in this thesis, out of the 608 genes up-regulated in liver tissue compared to HEPG2 (Tyakht *et al*., 2014), 596 genes could be identified (by matching gene names) in the reference gene set used in this thesis. Out of the 596 genes, 319 (53.5%) were expressed in worm-free HEPG2 (FPKM > 0) (data not shown).

5.3.4 Overall profiles of transcriptomes

PCA showed distinct clusters of each cell type. From the Figure 5.3 (all cell types), each cell type forms a distinct cluster regardless of whether the cells were co-cultured with schistosomules or without. Most of the transcriptional differences are therefore determined by cell type. The differences between worm-free and co-cultured cells were only seen when each cell type was clustered separately (Figure 5.3, HEPG2, HUVEC, GripTite). With such marked differences between cell types, samples from each cell type were considered as separate experiments.

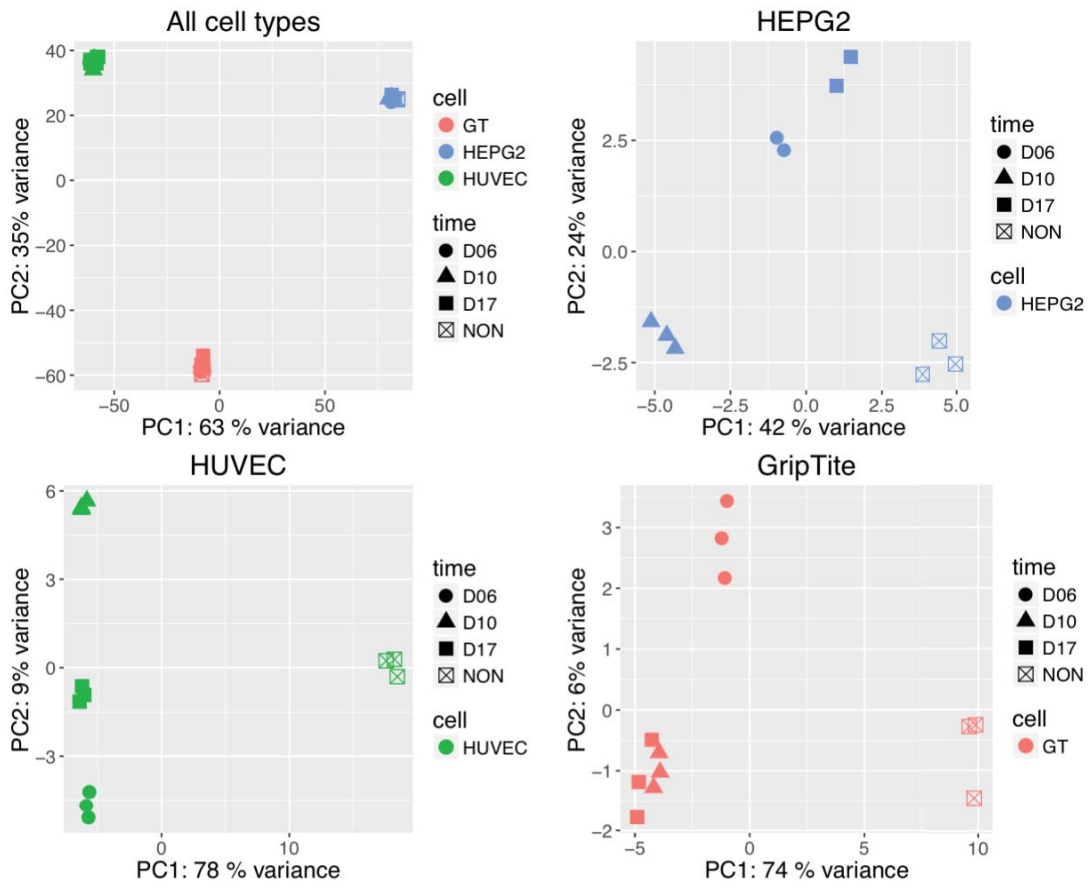

Figure 5.3 PCA from transcriptomes of all cell types, and separate for each cell type
Variation between samples shown as PCA plots when all samples (all three cell types) were considered together (All cell types). HEPG2, HUVEC, GripTite) variation within cell types as indicated by the plot titles. On each plot legend, 'time' refers to the age of schistosomules when the cells were collected. At the point of collection, all cells had been with schistosomules (regardless of schistosomule ages) for three days. NON: cells that were not co-cultured with schistosomules (worm-free cells).

When each cell type was treated individually, the samples that were co-cultured with worms formed distinct groups (filled shapes, Figure 5.3, HEPG2, HUVEC, GripTite), and all were separated from worm-free cells (crossed box, Figure 5.3 HEPG2, HUVEC, GripTite) along the first principal component axis (PC1) that explained 42-78% of transcriptional variation between samples. In contrast, the differences between cells that were exposed to the parasites of different time point were primarily separated along the PC2 axis which explained a much smaller percentage of variance (Figure 5.3). It could be seen here that the effect of time was relatively small compared to the effect of co-culture. Because of this distinct separation, I performed the analysis by placing cells into worm-free and co-cultured groups.

Next, I investigated the effect of the co-culture in each cell type separately; HUVEC, HEPG2, and lastly GripTite (modified HEK cells). Amongst the cell types used for this experiment, GripTite is the least similar to cell types that schistosomules would naturally encounter *in vivo*.

5.3.5 Differential expression between co-cultured vs. worm-free cells

5.3.5.1 HUVEC

Comparing co-cultured vs. worm-free in HUVEC, the number of differentially expressed genes was the greatest between similar comparisons in the other two cell types; 453 genes were down-regulated and 624 genes were up-regulated (adjusted p-value cut-off = 0.01, $\log_2$FC cut-off +/− 1) (Figure 5.4, Table S5.1, Table S5.2).

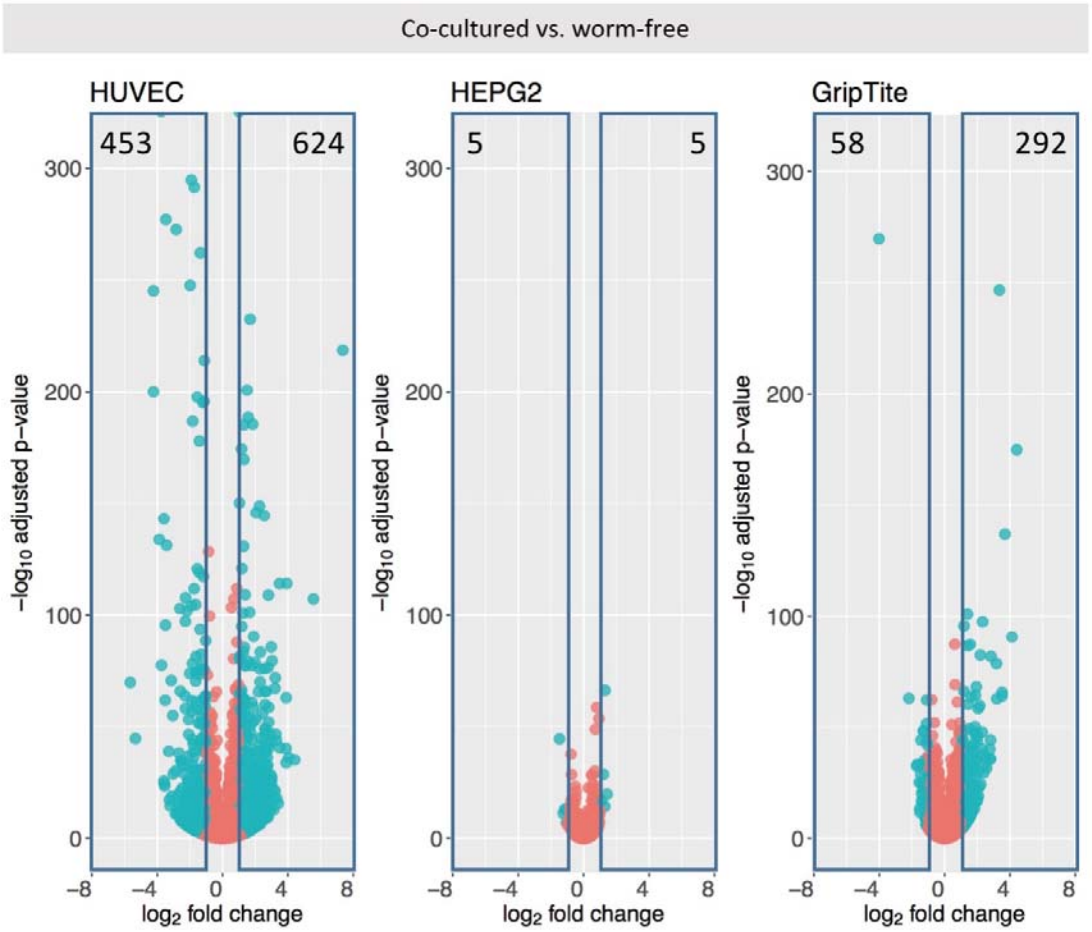

Figure 5.4 Volcano plots for pairwise comparison in each cell type between co-cultured* vs. *worm-free conditions

Log_2FC and adjusted p-value of differentially expressed genes in each cell type comparing between cells co-cultured with schistosomules *vs.* worm-free cells. Numbers at the top indicate the number of genes that pass cut-offs for calling genes differentially expressed with adjusted p-value < 0.01 and log_2FC +/−1 (shown as blue dots).

Endothelial cell adhesion molecules

Amongst the most down-regulated genes were those encoding adhesion proteins on endothelial cells. These are *selectin E* (*SELE*, 50.1 fold), *vascular cell adhesion molecule 1* (*VCAM1*, 40.3 fold), and *intercellular adhesion molecule 1* (*ICAM1*, 13.6 fold) (Figure 5.5, Figure 5.6, Table S5.2). In addition to the lower fold change of *ICAM1*, its expression in co-cultured HUVEC did not reduce to zero, unlike for *VCAM1* and *SELE* (Figure 5.5).

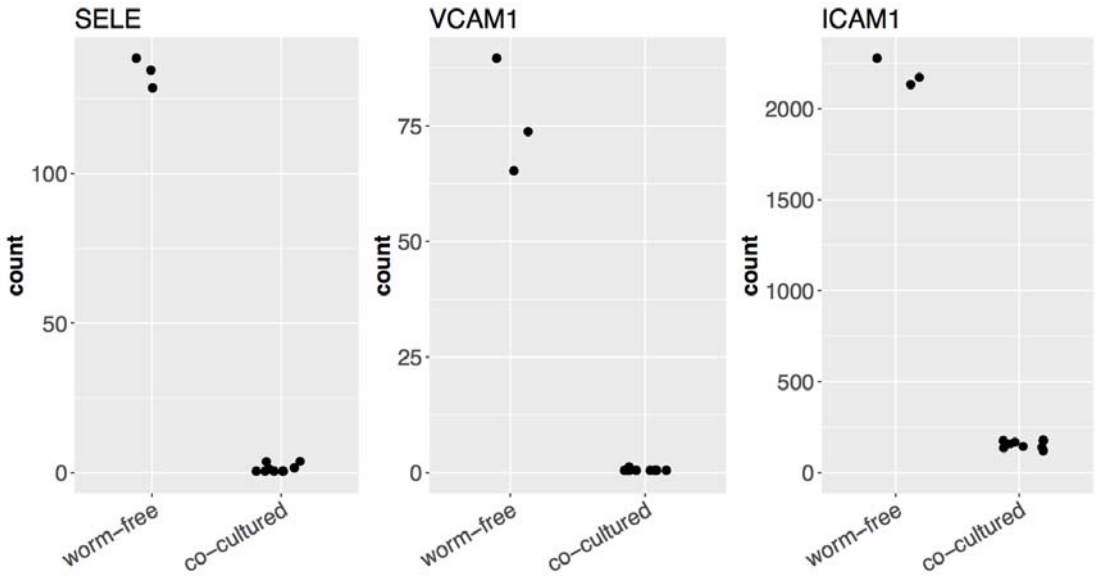

Figure 5.5 Expression profiles of SELE, VCAM1, and ICAM1

Expression of three genes encoding endothelial cell adhesion molecules that were down-regulated in co-cultured HUVEC. Each dot is one biological replicate. Y-axis represent normalised read counts. The lowest read counts of ICAM1 in co-cultured HUVEC were approximately 200 where as, for the SELE and VCAM1, the lowest read counts were close to zero. *SELE, selectin E; VCAM1, vascular cell adhesion molecule 1; ICAM1, intercellular adhesion molecule 1*.

These genes are involved in adhesion of circulating leukocytes and have important roles for recruitment of circulating immune cells to sites of injury or tissue damage. All three genes have been established as being regulated at transcription level by cytokines, LPS, and "other mediators of inflammation". In contrast, two other adhesion molecules *ICAM2* and *SELP* are not found to be regulated by the inflammation-related signals (Carlos & Harlan 1994). *ICAM2*, *SELP*, and other endothelial cell adhesion molecules (*SELL*, *PECAM1*, *MAdCAM1*) (Carlos & Harlan 1994) were not down-regulated in co-cultured HUVEC cells (Figure 5.6), suggesting that this is a specific suppression on inflammation-related adhesion molecules.

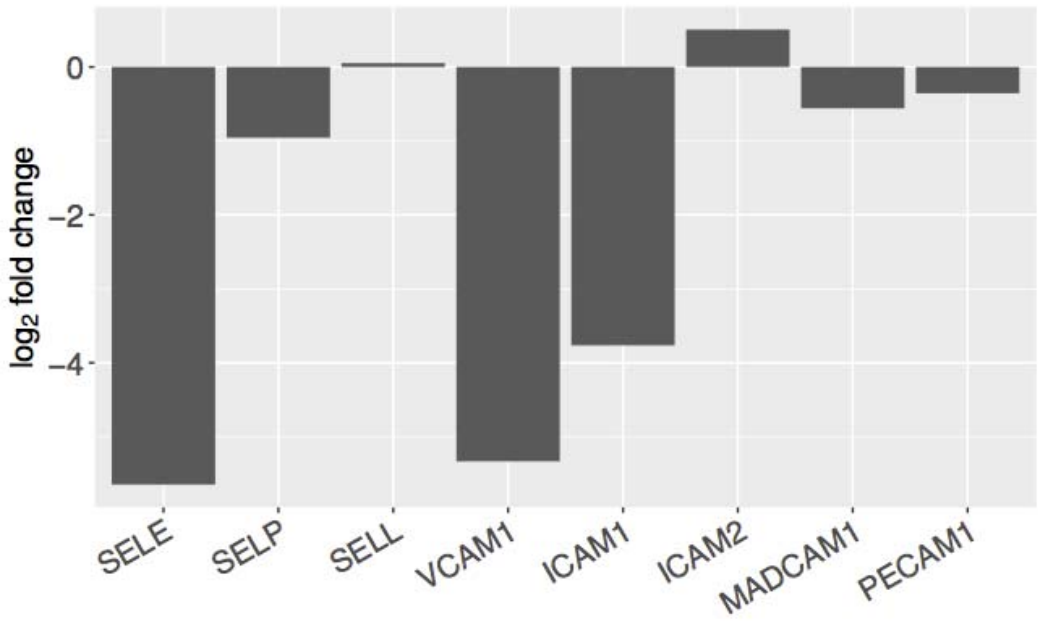

Figure 5.6 Expression of endothelial adhesion molecules between co-cultured* vs. *worm-free HUVEC

Log2FC of eight genes encoding endothelial adhesion molecules, comparing between HUVEC co-cultured with schistosomules and worm-free HUVEC. *SELE, selectin E; SELP, selectin P; SELL, selectin L; VCAM1, vascular cell adhesion molecule 1; ICAM1, intercellular adhesion molecule 1; ICAM2, intercellular adhesion molecule 2; MADCAM1, mucosal vascular addressin cell adhesion molecule 1; PECAM1, platelet/endothelial cell adhesion molecule 1*.

Extracellular matrix

There is also transcriptional evidence that the extracellular matrix (ECM) is remodelled; for example, up-regulated genes were enriched for the GO term *extracellular matrix organization* and down-regulated genes were enriched for the GO term *cell junction organisation* (Figure 5.7, Table S5.3). Furthermore, all 16 enriched pathways from the down-regulated genes can be related to extracellular matrix organisation (Figure 5.8, Table S5.4). Genes encoding different types of collagen chains, integrin subunits, and metalloproteinases were among the down-regulated as well as up-regulated genes (Table S5.1, Table S5.2). Schistosomules and adult *S. mansoni* have previously been shown to degrade ECM by a process that involve metalloproteinases (Keene *et al*., 1983; McKerrow *et al*., 1983). In this thesis, I have shown that ECM may also be affected as a result of gene expression changes in host cells that were exposed to schistosomules. Thereby, *S. mansoni* might affect the

ECM environment in two ways: by altering existing ECM, and by influencing host gene expression in the presence of the parasite.

Immune system

The massive down-regulation of the three leukocyte adhesion molecules (Figure 5.5) suggests anti-inflammatory profile of the co-cultured HUVEC. In addition to this, down-regulated genes were enriched in *immune system processes* (Figure 5.7, Table S5.3). Some of the down-regulated genes annotated with this GO term are involved in differentiation and activation of immune cells. For example, expression of *colony stimulating factor 1* was reduced by 3.5 fold, and that of *IL-32* was reduced by 2.5 fold. However, down-regulated genes also include inhibitors of the complement cascade, and the lower level would promote complement activation (Table S5.2). For example, gene expression of gene encoding CFH which functions to inhibit complement component 3 (C3) was reduced by 2.8 fold. The GO term *immune system process* was also enriched in the up-regulated genes (Figure 5.7), reflecting the nature of extensive connections in the immune system.

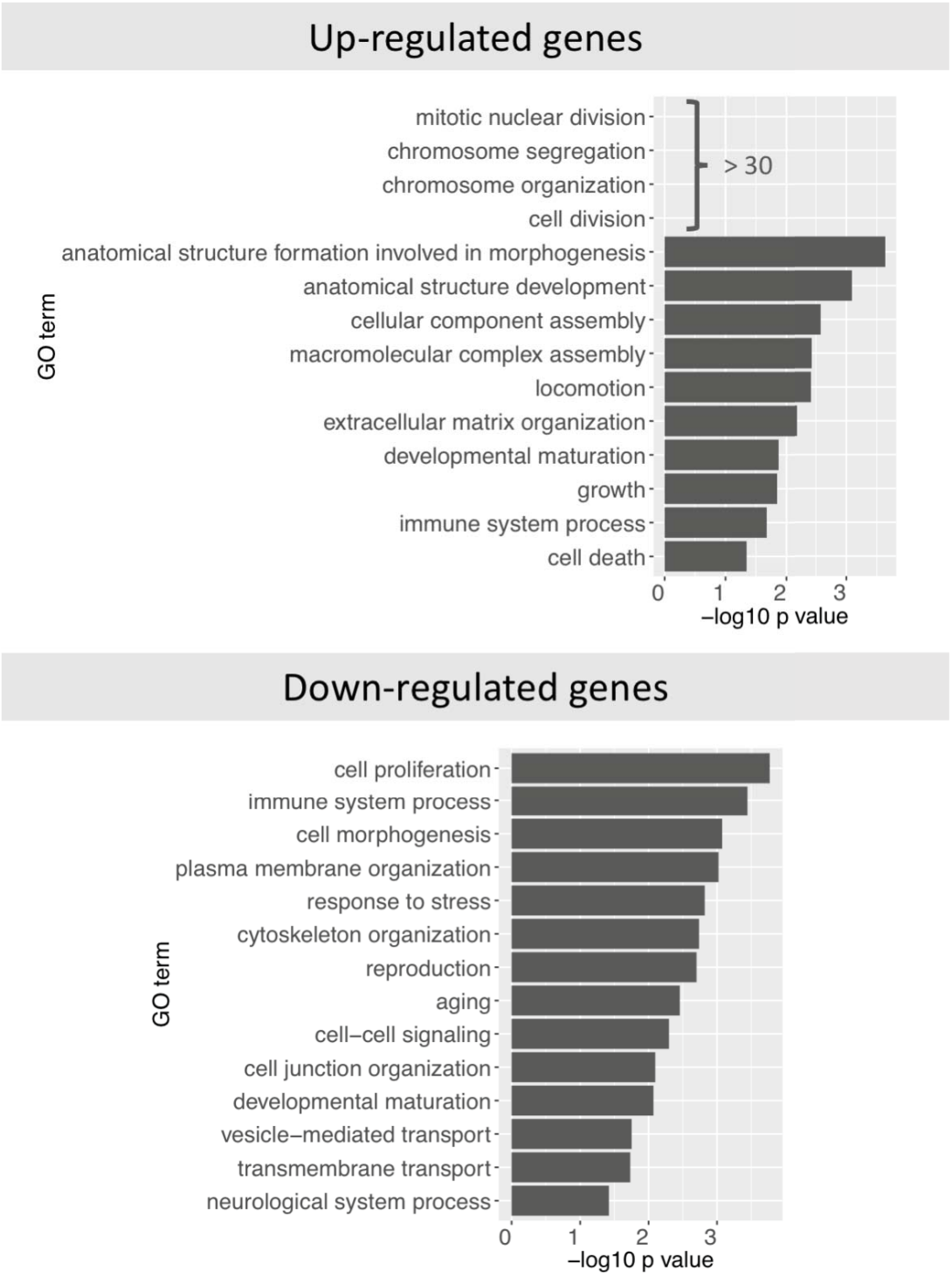

Figure 5.7 GO enrichment of genes differentially expressed in co-cultured HUVEC compared to worm-free HUVEC

Bar chart of enriched GO term (biological process) of up-regulated and down-regulated genes in co-cultured HUVEC. Expressed genes were used as enrichment reference background. Expressed genes were genes that showed at least some level of expression in at least one replicate of either co-cultured or worm-free condition (FPKM > 0).

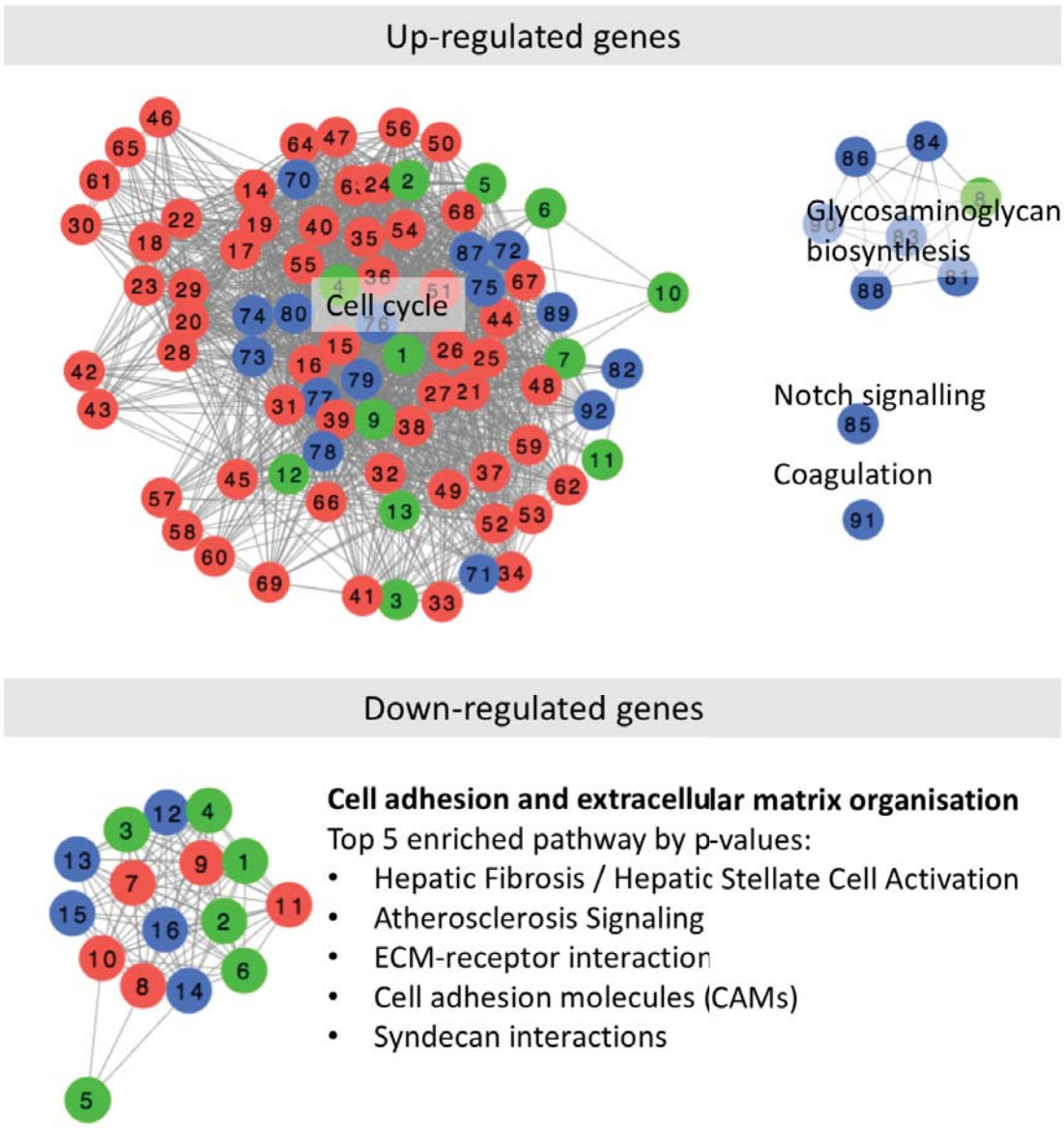

Figure 5.8 Pathway enrichment of genes differentially expressed in co-cultured HUVEC compared to worm-free HUVEC

Networks of enriched pathways from three pathway databases. Inputs to pathway enrichment analysis were differentially expressed genes between co-cultured and worm-free cells (HUVEC: adjusted p-value < 0.01, $\log_2$FC +/−1). Each node represents a pathway and the links between nodes indicate that the nodes share at least one gene. Colours indicate sources of the analysis results: red, Reactome; green, KEGG (via InnateDB interface); blue, IPA. Numbers on each node match the numbers in Table S5.4 which provide more details on each enriched pathway.

Coagulation

Coagulation was an enriched pathway in the up-regulated genes (Figure 5.8). The up-regulated genes in the pathway included two *plasminogen activator* genes (*PLAUR*

and *PLAT*) (Table S5.4). Plasminogen activators promote the formation of plasmin which degrades fibrin clots, and thereby reverse the blood clotting process. In addition, *thrombomodulin* (*THBD*) and *SERPIND1* were both up-regulated (Table S5.4) and their encoded proteins impede the coagulation cascade by reducing thrombin level (*THBD*), or by inhibiting thrombin functions (*SERPIND1*). Intriguingly, *Factor 3* (*F3*) gene whose protein product initiates blood coagulation cascade was also up-regulated (Table S5.4). However, the four genes that work to inhibit (or reverse) blood clotting were up-regulated by a larger effect size (Figure 5.9). In fact, *SERPIND1* was amongst the top 10 up-regulated genes in co-cultured HUVEC (up-regulated by 16.6 fold). Together this suggests inhibition of blood clotting process as well as degradation of fibrin clots once the clots have been formed.

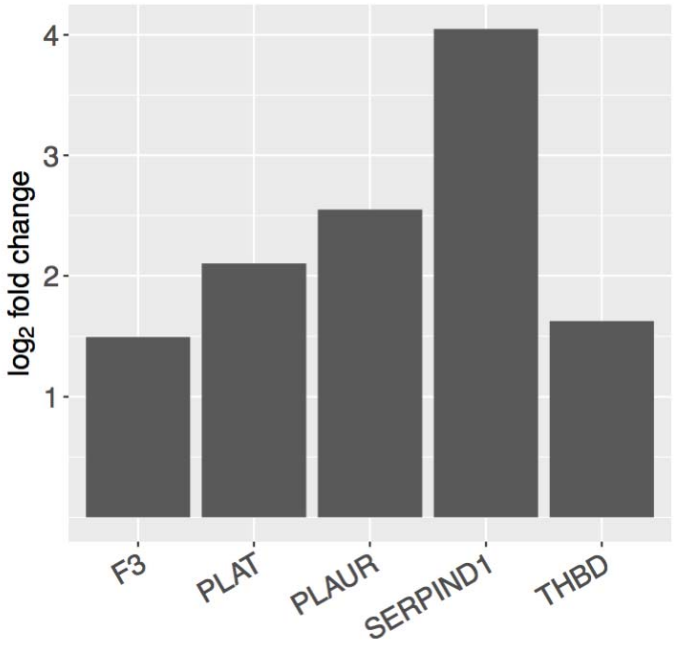

Figure 5.9 Coagulation-related genes up-regulated in co-cultured HUVEC
Coagulation-related genes from the IPA database that were up-regulated in co-cultured HUVEC compared to worm-free HUVEC. *F3, factor 3; PLAT, plasminogen activator (tissue type); PLAUR, plasminogen activator (urokinase receptor); SERPIND1, serpin peptidase inhibitor, clade D, member 1; THBD, thrombomodulin*.

Cell cycle

Cell cycle was strikingly affected in co-cultured HUVEC. This was reflected in both enriched GO terms and enriched pathways, most recognisably for the up-regulated genes. (Figure 5.7, Figure 5.8). The top four enriched GO terms were *chromosome*

segregation, *mitotic nuclear division*, *chromosome organization* and *cell division*, and the enrichment of these GO terms were strongly significant (Figure 5.7). Many pathways related to cell cycle were enriched in the up-regulated genes (Figure 5.8). Up-regulated were genes encoding key regulatory functions for cell cycle such as *cyclins* and cyclin-dependent *kinases* (Table S5.4). However, in down-regulated genes, *cell proliferation* was also the most significantly enriched GO term (Figure 5.7). Cell cycle process is tightly controlled by many genes and check points; therefore, the presence of genes related to cell cycle in both up-regulated and down-regulated genes may not be surprising. Furthermore, up-regulated and down-regulated genes consist of both genes that promote cell cycle progression and genes that inhibit the progression (Table S5.4). As a result, interpreting phenotypic outcome for cell proliferation is challenging.

Notch signalling

In addition to the effect on cell cycle control and mitosis, up-regulated genes in co-cultured HUVEC were enriched in the *Notch signalling pathway* (Figure 5.8). Notch signalling has been described as a link between innate and adaptive immunity (Ito *et al*., 2012), as well as regulating cell-cell interaction between adjacent cells in a process involving differentiation, proliferation, and apoptosis (Chigurupati *et al*., 2007). Genes in the *Notch signalling pathway* that were up-regulated were *Notch4 receptor*, *JAG1* (ligand of multiple Notch receptors), *DDL4* (ligand of Notch1 and 4, and regulator of endothelial cell proliferation), and transcriptional repressors downstream of Notch signalling *HEY2*, *HES7*, *HEY1* (Ranganathan *et al*., 2011) (Table S5.4). With these changes covering genes from Notch receptors to Notch-regulated transcriptional factors, Notch signalling and its role in controlling cell-cell interaction and cell proliferation appears to be affected.

HUVEC conclusion

Taken together, the HUVEC responses to the schistosomules primarily affected cell cycle and extracellular matrix organisation. Genes related to immune responses were affected but resulting phenotypes were unclear. However, changes in coagulation pathway genes suggest reduction of blood clotting, and down-regulation of leukocyte adhesion molecules suggests an anti-inflammatory profile.

5.3.5.2 HEPG2

Top differentially expressed genes

The effect of the co-culture on HEPG2 cells was the smallest among the three cell types tested. Only five genes were up- and five genes were down-regulated (log_2FC cut-off +/−1) (Table 5.1). Strikingly, the five down-regulated genes included four genes whose products can be associated with anti-microbial peptide or innate responses to bacterial infection such as recognition of LPS, and are listed as follows. Haptoglobin (2.8-fold down-regulated) is an antimicrobial peptide that also binds to free heme reducing iron availability to pathogens (Yang *et al*., 1983). A form of Kininogen 1 (2.4-fold down-regulated) is involved in coagulation and production of bradykinin which is an antimicrobial peptide (Hofman *et al*., 2016). Hepcidin (2.2 fold down-regulated), in addition to being an antimicrobial peptide, functions in iron homeostasis of liver and intestine (Ganz and Nemeth, 2012). Finally, LPS-binding protein (2-fold down-regulated) is involved in acute responses to bacterial LPS (Bochkov *et al*., 2002). Together, down-regulation of these genes suggests that part of the innate immune response is suppressed in co-cultured HEPG2 (Table 5.1). Up-regulated genes however did not show clear relevance to innate immune response but instead include genes involved in modification of collagen, cell-cell interaction, cell migration, proliferation and metabolism (Table 5.1).

Table 5.1 Top differentially expressed genes in HEPG2

	Log_2FC (Co-cultured / worm-free)	Adjusted p-value	Product name	Product description
Top five up-regulated genes				
ENSG00000198756	1.43	2.00E-20	COLGALT2	collagen beta(1-O)galactosyltransferase 2
ENSG00000131746	1.31	6.13E-67	TNS4	tensin 4
ENSG00000064300	1.30	6.41E-15	NGFR	nerve growth factor receptor
ENSG00000117394	1.21	2.89E-29	SLC2A1	solute carrier family 2 (facilitated glucose transporter); member 1
ENSG00000183196	1.10	2.09E-17	CHST6	carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 6
Top five down-regulated genes				
ENSG00000257017	-1.47	3.03E-45	HP	haptoglobin
ENSG00000113889	-1.24	7.77E-12	KNG1	kininogen 1
ENSG00000105697	-1.15	1.40E-13	HAMP	hepcidin antimicrobial peptide
ENSG00000132854	-1.10	9.36E-14	KANK4	KN motif and ankyrin repeat domains 4
ENSG00000129988	-1.04	9.03E-08	LBP	lipopolysaccharide binding protein

To increase sensitivity for HEPG2, I reduced the log_2FC cut-off from +/−1 (2-fold change) to +/−0.5 (1.4-fold change). With this relaxed cut-off, 193 genes were detected as up-regulated (Table S5.5), and 137 genes were down-regulated (Table S5.6). The genes were used as inputs for GO term enrichment (Figure 5.10, Table S5.7) and pathway enrichment (Figure 5.11, Table S5.8). The results were consistent with the functions of top five up-regulated and top five down-regulated genes discussed previously but with additional biological processes identified; such as *cell differentiation* GO term and *ECM-receptor interaction* pathway for up-regulated genes, and with *metabolic processes* and *complement and coagulation pathway* in down-regulated genes (Figure 5.10, Figure 5.11).

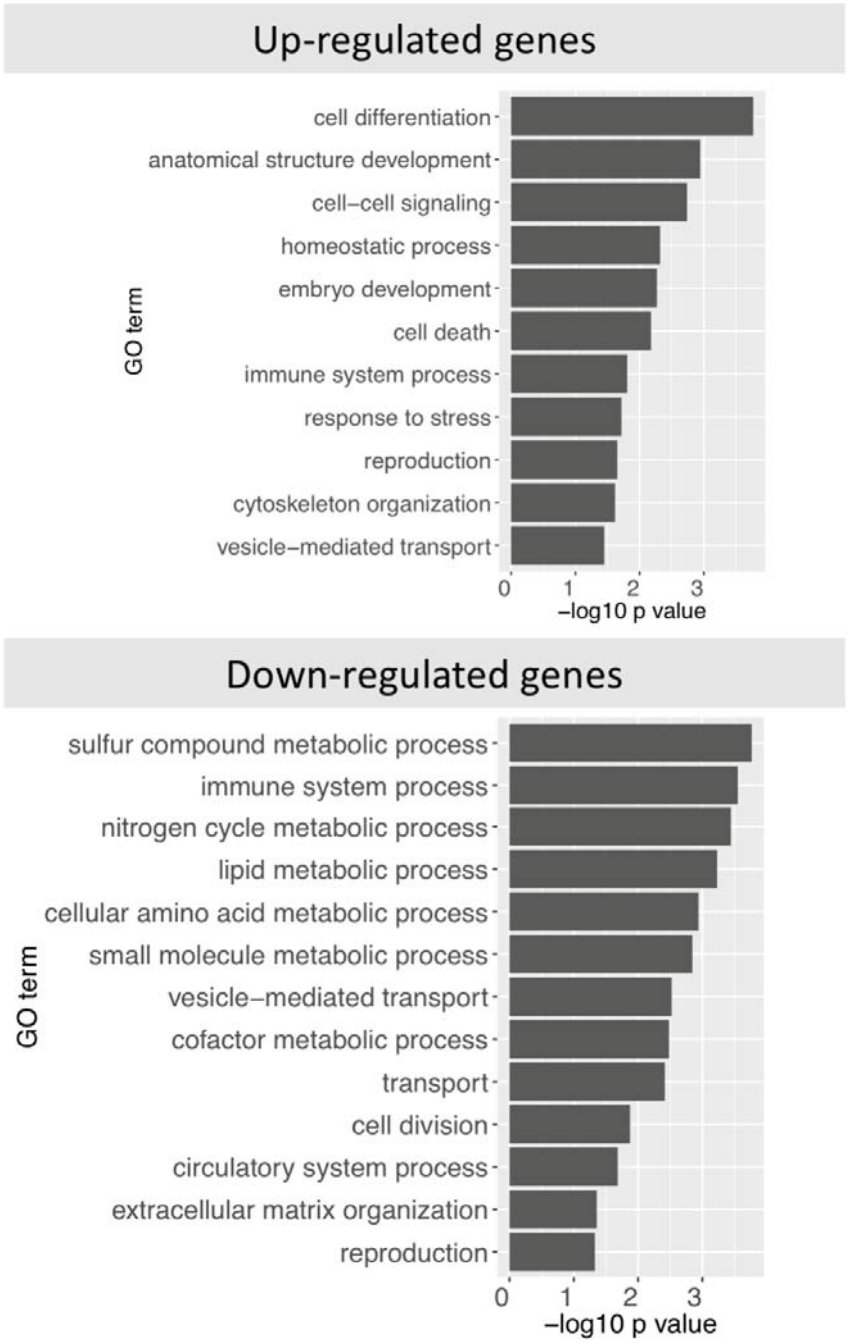

Figure 5.10 GO enrichment of genes differentially expressed in co-cultured HEPG2 compared to worm-free HEPG2

Bar chart of enriched GO terms (biological process) of up-regulated and down-regulated genes in co-cultured HEPG2. Expressed genes were used as enrichment reference background. Expressed genes were genes that showed at least some level of expression in at least one replicate of either co-cultured or worm-free condition (FPKM >0).

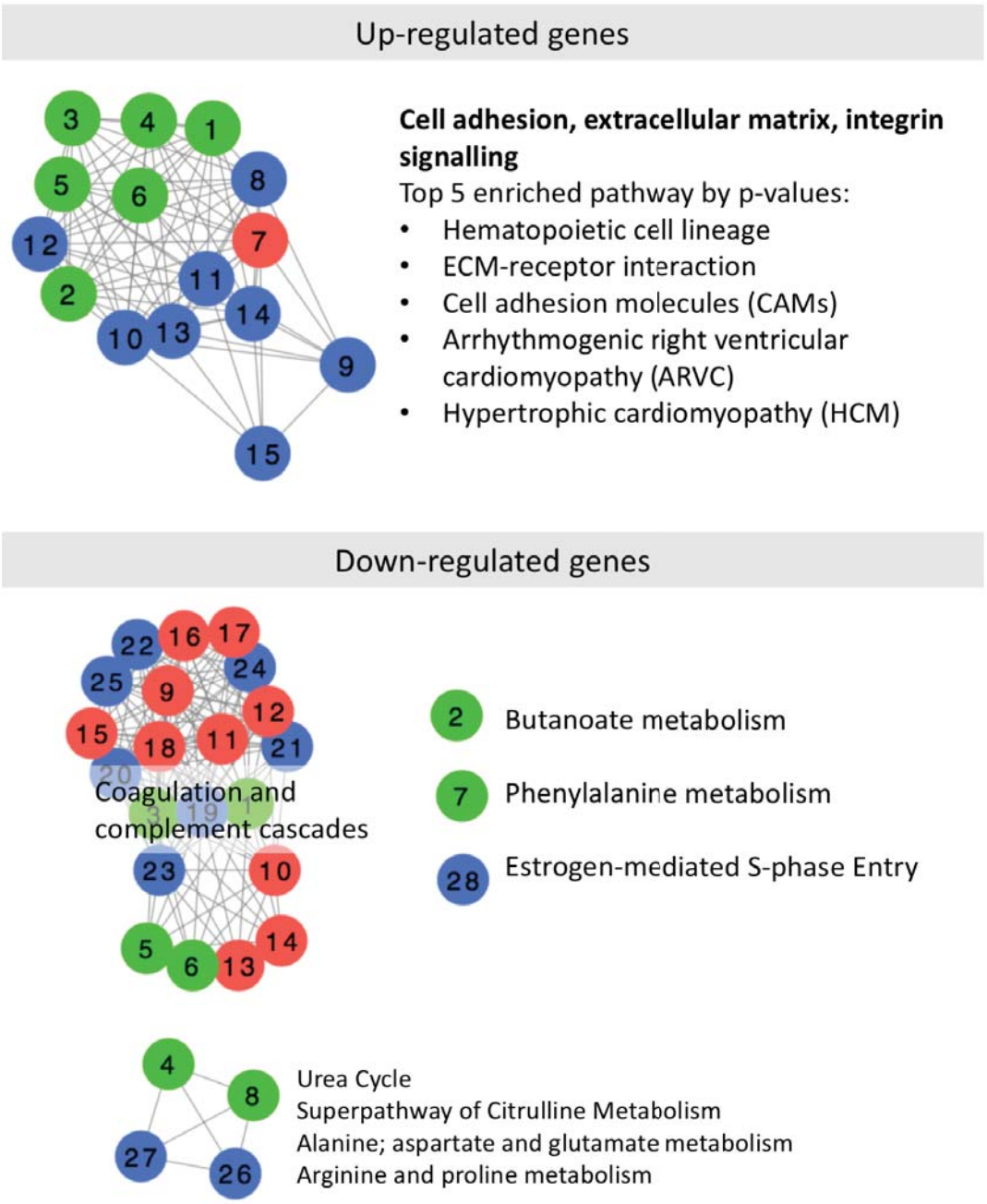

Figure 5.11 Pathway enrichment of genes differentially expressed in co-cultured HEPG2 compared to worm-free HEPG2

Networks of enriched pathways from three pathway databases. Inputs to pathway enrichment analysis were differentially expressed genes between co-cultured and worm-free cells (HEPG2: adjusted p-value < 0.01, $\log_2$FC +/−0.5). Each node represents a pathway and the links between nodes indicate that the nodes share at least one gene. Colours indicate source of the analysis results: red, Reactome; green, KEGG (via InnateDB interface); blue, IPA. Numbers on each node match the numbers in Table S5.8 which provide more details on each enriched pathway.

Overview of up-regulated genes

The GO terms enriched amongst up-regulated genes included *cell differentiation*, *cell death*, *development*, and *cell-cell signalling*. These are also reflected in the results of pathway enrichment-analysis where *ECM-receptor interactions* and other cell-cell interaction pathways were highlighted (Figure 5.10, Figure 5.11). Many of the genes responsible for the enrichment encoded integrins (Table S5.7, Table S5.8). Integrins are localised at cell membrane and provide a link between the cytoskeleton of a cell and the ECM. This suggests increased interactions of HEPG2 cells with their extracellular matrix environment, which contrasts with the response of HUVEC. In co-cultured HUVEC, similar pathways and GO terms were enriched in down-regulated genes (Figure 5.7, Figure 5.8). Toward the end of this chapter, I explore changes of genes in *ECM organisation* pathway in more detail, comparing between three cell types.

Overview of down-regulated genes

The down-regulated genes fall into two main types according to functional enrichment: metabolic processes, and immune response and circulation processes (Figure 5.10, Figure 5.11). Metabolic processes include metabolism of amino acids (urea cycle, and phenylalanine metabolism pathways), lipid, and sulfur compounds. In particular, three genes out of six genes in the urea cycle were down-regulated and potentially could affect metabolism of amino acids (Table S5.8). Furthermore, some of the genes encoding enzymes in urea cycle have roles in immune responses - *arginase 1* - and was down-regulated by 1.6-fold (Table S5.6). Immune response and circulation processes affected in co-cultured HEPG2 were genes in the complement and coagulation cascade (Figure 5.11). These are directly relevant to the infection with *S. mansoni* and are explored further as follows.

Coagulation

Regarding the coagulation, *fibrinogen alpha, beta, and gamma chain* were all down-regulated (fold changes between 1.6-1.7) (Table S5.6). Fibrinogens are required for formation of blood clot and are converted by thrombin into insoluble fibrin strand (Mebius *et al*., 2013). In addition to fibrinogen, genes encoding serine protease inhibitors, coagulation factor 7 and factor 12 that are situated at the beginning of coagulation cascade were down-regulated (Table S5.6). Factor 7 is responsible for the

initiation of extrinsic coagulation pathway (from tissue factor released from damaged tissue), and factor 12 is responsible for the initiation of intrinsic blood coagulation pathway (contact activation from damaged blood vessel surface revealing charged surface) (Renne *et al*., 2012). On the other hand, the down-regulated genes also included genes that regulate coagulation such as *serine proteinase inhibitor SERPINC1* and *SERPINF2* (Table S5.6); meanwhile, the up-regulated genes include genes that lead to degradation of blood clots, *PLAU* and *PLAUR* (Table S5.5). Together, changes of genes in this pathway suggest an anti-coagulation profile, which is in contrast to responses to microbes where coagulation is often activated (Esmon *et al*., 2011).

Complement cascades

For the complement cascade, genes that were down-regulated in co-cultured HEPG2 fell into the alternative pathway, classical pathway and the formation of membrane attack complex (genes encoding CFB, C1R, C1S, C4B, C5, C8B, C8G) (Table S5.8). Both pathways are capable of killing the parasites (Marikovsky *et al*., 1986; Ruppel *et al*., 1983; Santoro *et al*., 1979). However, and interestingly, C3 that is essential for complement cascade progression was not affected in co-cultured HEPG2. Nevertheless, C5 and C8 which are required for downstream steps of all complement pathways (after the activation of C3) were both down-regulated (Table S5.8).

HEPG2 conclusion

In summary, genes from HEPG2 that were most affected by the co-culture had roles in ECM organisation, cell-ECM interaction, metabolism, and immune responses. From the gene expression data and functional enrichment, it appeared that the coagulation pathway was impeded - genes that initiate the cascade were down-regulated, genes that lead to degradation of fibrin clots were up-regulated. For the complement cascade, initiators of the classical pathway were down-regulated as well as genes that are shared between all three complement pathways.

5.3.5.3 GripTite

GripTite, modified HEK293 cells, were used in this experiment to represent cells that the parasite would not normally encounter in natural infections. When co-cultured with schistosomules, the number of differentially expressed genes in GripTite cells fell in between the numbers seen for endothelial cells and HEPG2 cells. There was a

skew toward genes being up-regulated in co-cultured cells, with 292 genes up-regulated (Table S5.9) and only 58 genes down-regulated (Table S5.10) (log_2FC cut-off at +/−1, adjusted p-value cut-off at 0.01; Figure 5.4).

Neuronal signalling

Based on GO annotations, the differentially expressed genes in co-cultured GripTite cells are involved in signalling, cell differentiation, developmental control, metabolism, and carrier for ion transport (Figure 5.12, Table S5.11). The top 10 up-regulated genes are all involved in receptor signalling and transcriptional regulation (Table S5.9). Some of these top 10 up-regulated genes are related to nervous system functions; for example, *G protein-coupled receptor 3* (*GPR3*) and *activity-regulated cytoskeleton-associated protein* (*ARC*) both function in neurons (Dynes and Steward, 2007; Kumar *et al*., 2015).

JAK-STAT, MAPK, NF-κB signalling

The nature of top 10 up-regulated genes were also supported in pathway enrichment based on KEGG, Reactome, and IPA databases where the majority of enriched pathways are related to receptor signalling including *JAK-STAT signalling pathway*, *MAPK pathway*, and *NF-κB signalling* (Figure 5.13, Table S5.12). All of these are signalling pathways that regulate transcription, cell survival, proliferation, differentiation, and immune responses (Dhillon et al., 2007; Hoesel and Schmid, 2013; Rawlings, 2004). In addition, the pathways are known to have roles in cancer progression and metastasis via regulation of cell proliferation, and survival (Dhillon et al., 2007; Hoesel and Schmid, 2013; Rawlings, 2004). GO term enrichment of down-regulated genes returned processes downstream of these pathways, such as *cell differentiation* and *anatomical structure development* (Figure 5.12), indicating the outcome that may be affected by the signalling.

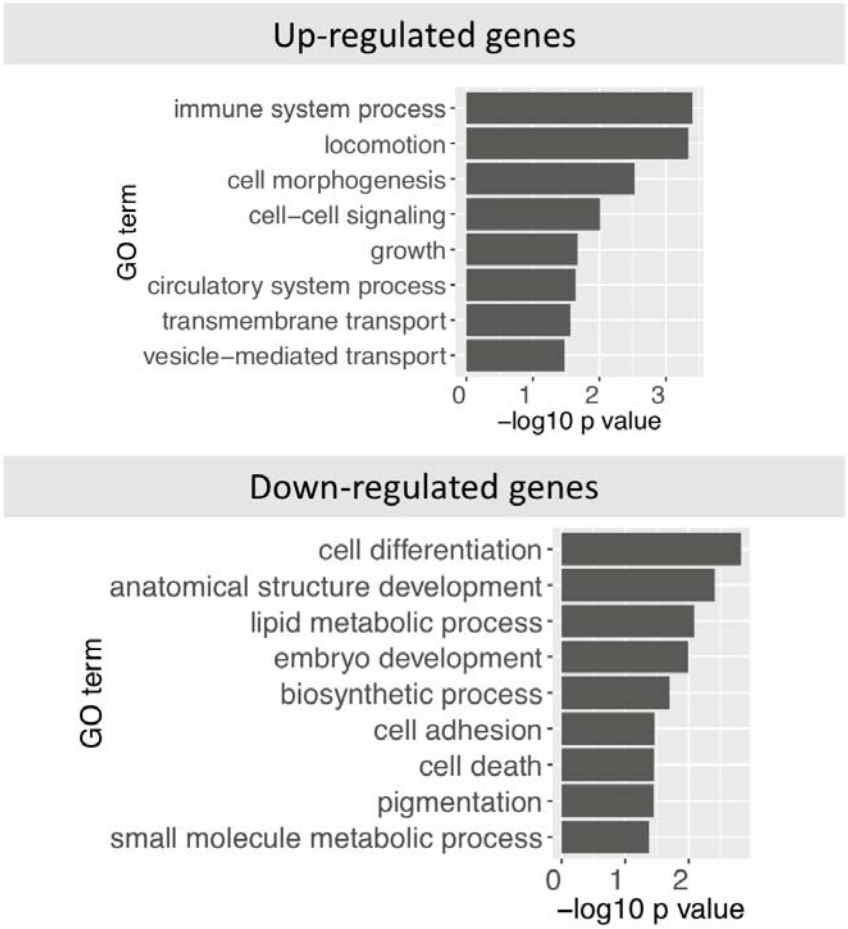

Figure 5.12 GO enrichment of genes differentially expressed in co-cultured GripTite compared to worm-free GripTite

Bar chart of enriched GO terms (biological process) of up-regulated and down-regulated genes in co-cultured GripTite. Expressed genes were used as enrichment reference background. Expressed genes were genes that showed at least some level of expression in at least one replicate of either co-cultured or worm-free condition (FPKM > 0).

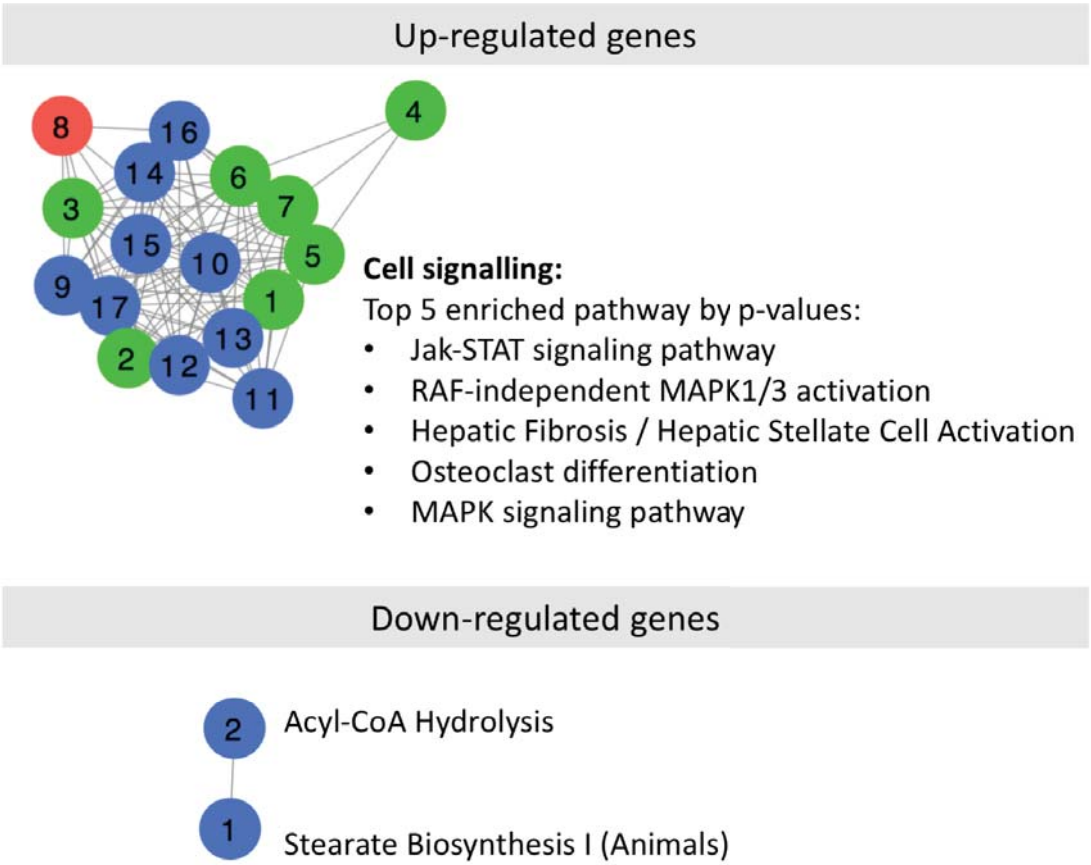

Figure 5.13 Pathway enrichment of genes differentially expressed in co-cultured GripTite compared to worm-free GripTite

Networks of enriched pathways from three pathway databases. Inputs to pathway enrichment analysis were differentially expressed genes between co-cultured and worm-free cells (GripTite: adjusted p-value < 0.01, $\log_2$FC +/−1). Each node represents a pathway and the links between nodes indicate that the nodes share at least one gene. Colours indicate sources of the analysis results: red, Reactome; green, KEGG (via InnateDB interface); blue, IPA. Numbers on each node match the numbers in Table S5.12 which provide more details on each enriched pathway.

Immune system process

Genes involved in immune responses were differentially expressed, particularly those with pro-inflammatory functions - contrary to the responses of HUVEC and HEPG2 cells where anti-inflammatory profiles are observed. In co-cultured GripTite, *immune system process* was the most significantly enriched GO term amongst the up-regulated genes (Figure 5.12, Table S5.11). Amongst immune-related genes, those encoding the following protein products were the most differentially expressed (Figure 5.14, Table S5.9, Table S5.11): TAC1 being a antimicrobial peptide (Sun and Bhatia, 2014);

RUNX1 regulating expression of IL2 and promoting T cell proliferation (Ono *et al*., 2007); IL6R involved in the acute phase response (Heinrich *et al*., 1990); FOS controling cell proliferation (Shaulian and Karin, 2001); EPHA2 being a receptor involved in signalling; EGR3 involved in leukocyte development (Li *et al*., 2012); EGR1 regulating early immune response gene expression (Decker *et al*., 2003; McMahon and Monroe, 1996); and CD44 involved in lymphocyte activation and angiogenesis (van Royen *et al*., 2004; Stefanová *et al*., 1989). However, genes whose product might suppress immune responses or have both pro- and anti-inflammatory function were also up-regulated such as genes encoding ANXA3 (possible anticoagulant) (Tait *et al*., 1991), and ANXA1 (down-regulated immune responses) (Wallner *et al*., 1986).

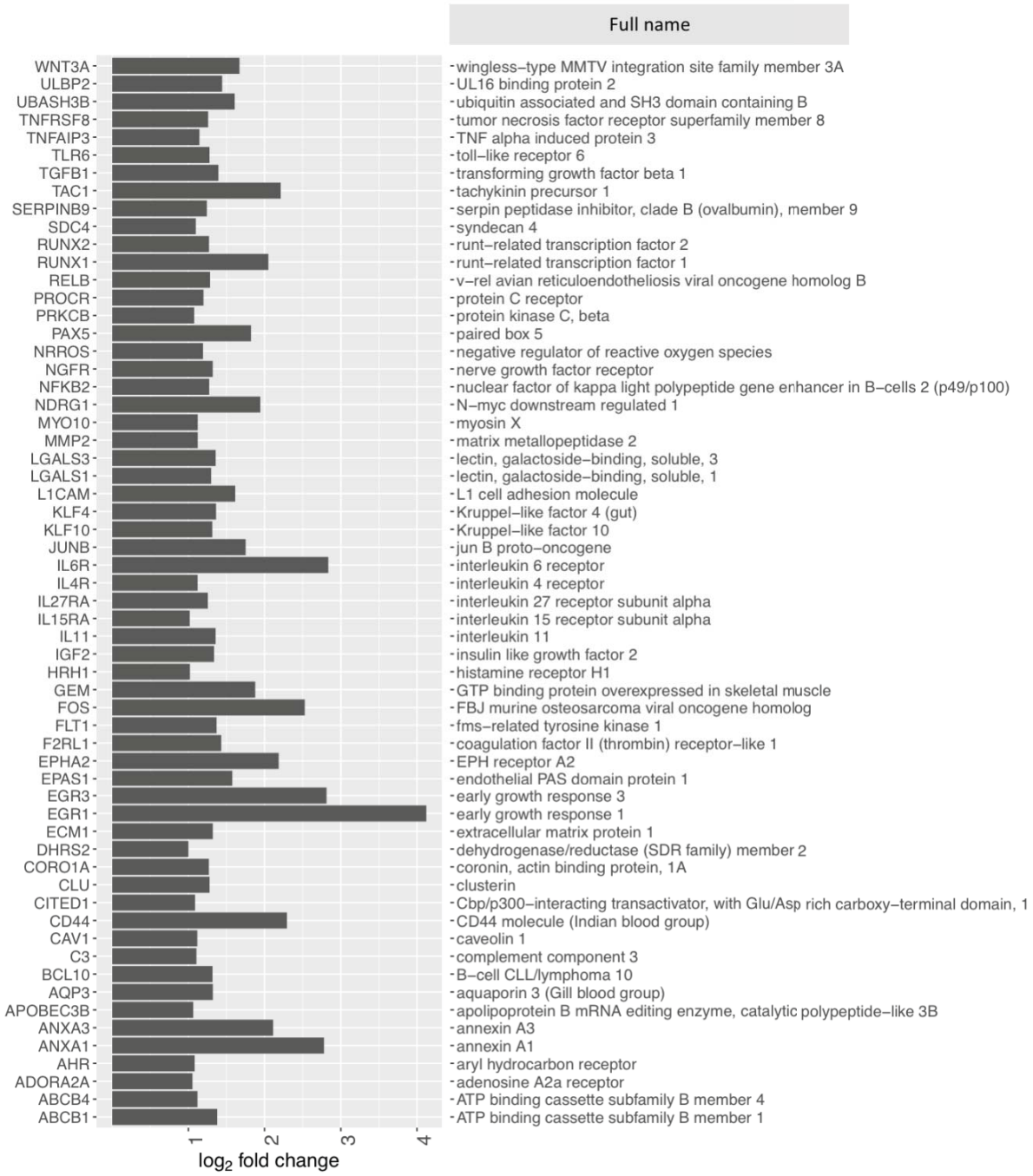

Figure 5.14 Immune responses-related genes up-regulated in co-cultured GripTite

Log_2FC of genes that are in GO term immune system process and were up-regulated in co-cultured GripTite compared to worm-free GripTite.

Griptite summary

In summary, GripTite cells co-cultured with schistosomules seem to be altered in genes related to signalling processes and the immune responses. Multiple up-regulated genes can be related to signalling involved in cell proliferation. Amongst the down-regulated genes, metabolic pathway of acetyl-CoA and lipid might be

affected. However, the results were not consistent when different methods of enrichment analyses were considered.

5.3.6 Pathways between cell types

From the previous section, some common features emerged between co-cultured cell types. Specifically, some of the pathways were affected in all three cell types, although different genes in the pathways were affected. In this final section, two pathways were chosen to compare the effect of the co-culture between cell types: first, the *extracellular matrix organisation* pathway, affected in either up-regulated or down-regulated genes in all three cell types; and second, the *coagulation and complement cascades* affected in HUVEC and HEPG2 cells that is directly relevant to *S. mansoni* infections.

5.3.6.1 Extracellular matrix organisation

Among the differentially expressed genes, the largest changes for both up- and down-regulated genes were in HUVEC. Comparing between cell types and considering genes with large fold changes (denser colours on the heatmap in Figure 5.15), *ICAM1*, and *BMP4* were down-regulated in co-cultured HUVEC, but were up-regulated in HEPG2 (Figure 5.15).

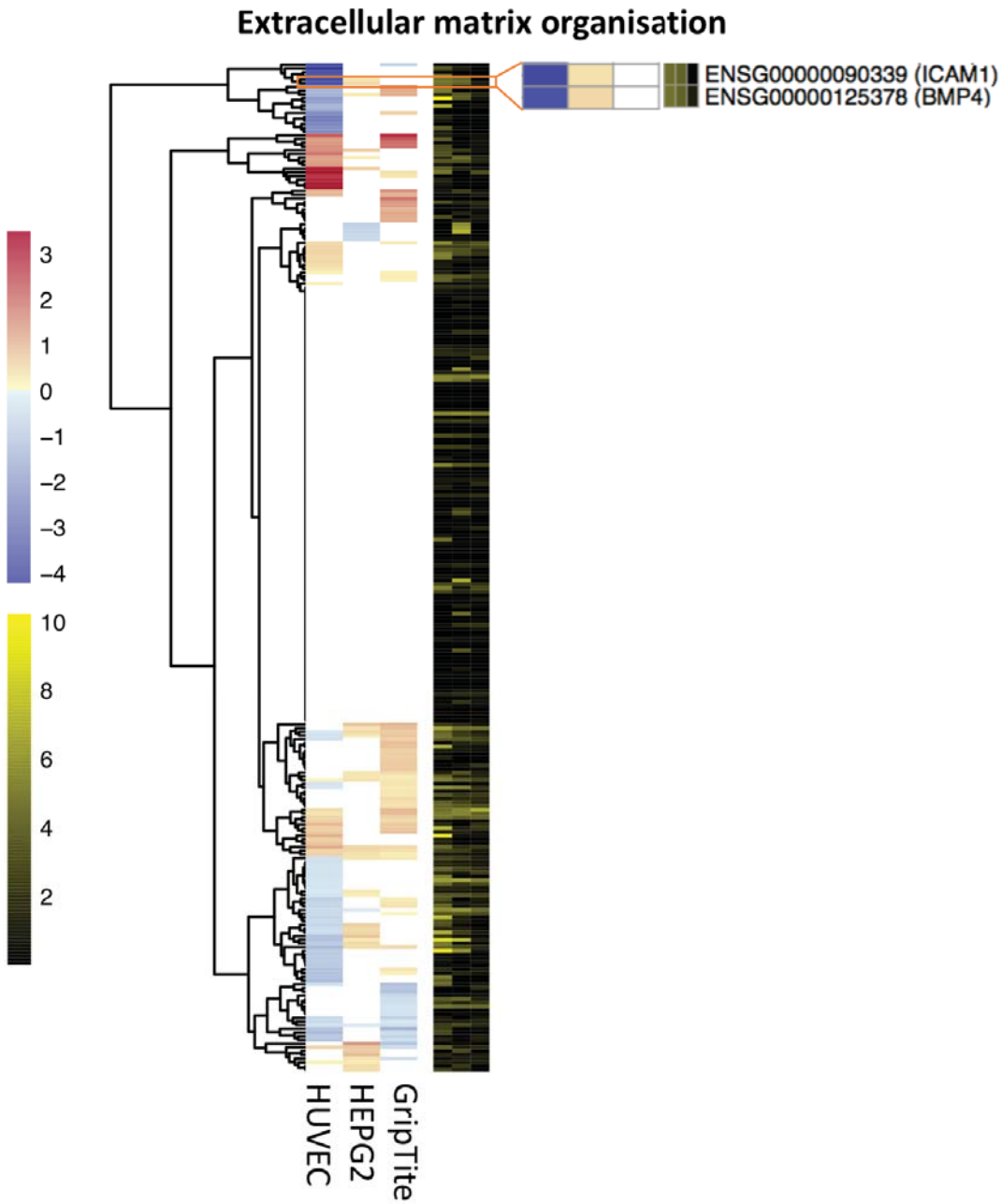

Figure 5.15 Log_2FC of genes in* extracellular matrix organisation *pathways

Genes in Reactome pathway *extracellular matrix organisation* and their differential expression in HUVEC, HEPG2, and GripTite compared between co-cultured cells and worm-free cells within each cell type. Colour map on red-blue spectrum indicates log_2FC from pairwise comparison of co-cultured *vs.* worm-free conditions for each cell type. Colour map on yellow-black spectrum indicates expression level (log_{10} of the highest FPKM). Only genes that were expressed in at least one replicate (FPKM > 0) in all cell types were included in the heatmap. The sections of the heatmap where no colour was displayed for the log_2FC are genes that were not differentially expressed.

ICAM1 and BMP4

ICAM1 is required for adhesion of leukocytes (Carlos and Harlan, 1994), while BMP4 induces activation of dendritic cells (Martínez *et al*., 2011). The down-regulation of these genes in co-cultured HUVEC suggests a shift toward an anti-inflammatory immune profile. However, for liver, ICAM1 has a role in liver regeneration (Selzner *et al*., 2003). Whereas BMP4 is essential for liver tissue repair following injury (Oumi *et al*., 2012), even though it has been also described as inhibiting liver regeneration (Do *et al*., 2012). Although the parasites were unlikely causing tissue damage in the *in vitro* set up, the up-regulation in liver might infer preparation for tissue repair.

5.3.6.2 Coagulation and complement cascades

Given that most of the genes in complement and coagulation pathway are expressed mainly in liver hepatocytes, it is reassuring that the coagulation and complement pathways were down-regulated in co-cultured HEPG2 but not in the other two cell types. However, some genes in the pathway (such as *CLU*, *F2R*, *FXII*, *PLAUR*, and *PLAU*) were also expressed in other tissues and may provide insight on the effect of co-culture between cell types (Figure 5.16).

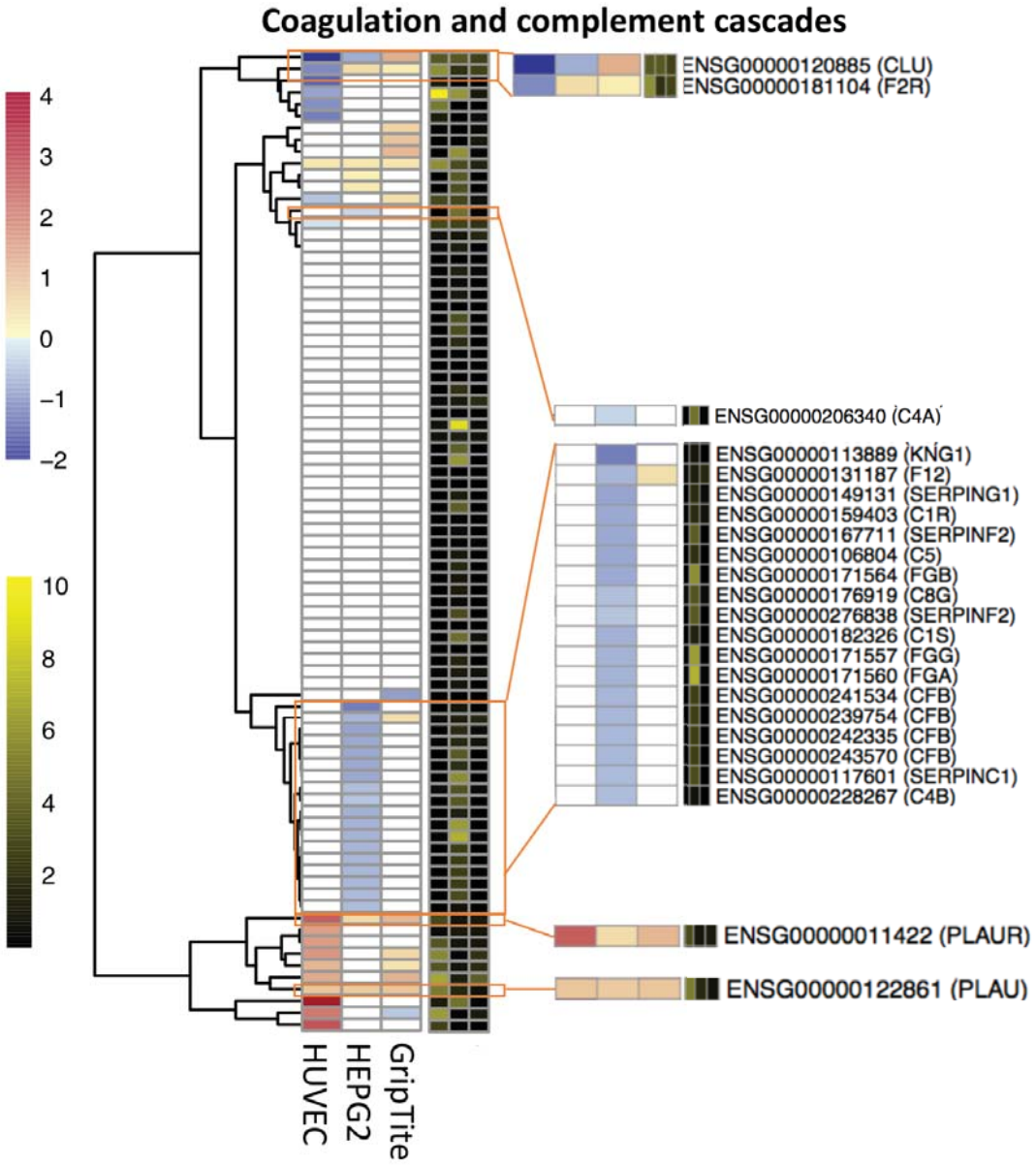

***Figure 5.16 Log$_2$FC of genes* in coagulation and complement cascade**

Genes in KEGG pathway *coagulation and complement cascades* and their differential expression in HUVEC, HEPG2, and GripTite compared between co-cultured cells and worm-free cells within each cell type. Colour map on red-blue spectrum indicates log$_2$FC from pairwise comparison of co-cultured *vs.* worm-free conditions for each cell type. Colour map on yellow-black spectrum indicates expression level (log$_{10}$ of the highest FPKM). Only genes that were expressed in at least one replicate (FPKM > 0) in all cell types were included in the heatmap. The sections of the heatmap where no colour was displayed for the log$_2$FC are genes that were not differentially expressed.

CLU (clusterin) and F2R (factor 2 receptor)

Considering genes with high expression levels in all three cell types, *clusterin* (*CLU*) and *coagulation factor 2 receptor* (*F2R*) were affected differently between the three cell types. Both genes were down-regulated in co-cultured HUVEC (Figure 5.16); whereas clusterin was also down-regulated in HEPG2 cells but up-regulated in GripTite (Figure 5.16). *F2R* was down-regulated in both co-cultured HEPG2 and GripTite cells (Figure 5.16). Both genes are involved in the coagulation and complement cascades but also regulate other processes such as cell proliferation and apoptosis (Reinhardt et al., 2012; Santilli et al., 2003). *Clusterin* also modulates the *NF-κB signalling pathway* (Santilli *et al*., 2003) and this may explain their up-regulation in co-cultured GripTite (where NF-κB is among the pathways up-regulated). F2R is a GPCR receptor that binds to thrombin and is thought to have a role in platelet activation, but also involved in vascular remodelling (Reinhardt *et al*., 2012). Down-regulation in HUVEC may prevent these processes, but the gene might have different functions in the other two cell types.

PLAU PLAUR (Plasminogen Activator, Urokinase and its receptor)

Plasminogen Activator, Urokinase (*PLAU*) and *Plasminogen Activator, Urokinase Receptor* (*PLAUR*) were up-regulated in co-cultured cells of all three cell types (Figure 5.16). Functions of these genes were discussed previously in HUVEC section regarding degradation of blood clot. The two genes together promote plasmin formation and later plasmin which is required to degrade blood clots. In addition, the genes are also involved in extracellular matrix remodelling (Gilkes *et al*., 2014; Martinez-Hernandez *et al*., 2011). The consistent pattern between cell types suggests that this could be a generic response to schistosomules. It further suggests that the regulation of coagulation in *S. mansoni* infections could provide a vulnerability with potential for therapeutic exploitation.

5.4 Discussion

5.4.1 Overview

In this chapter, I studied transcriptomic changes in human-derived cells co-cultured with schistosomules. The aim was to gain further insights into host responses to the parasites and how the responses might affect outcome of the infection. I have shown

that the gene expression of the co-cultured HUVEC and HEPG2 exhibited anti-inflammatory and anti-coagulation profiles, whereas changes in co-cultured GripTite may suggest activation of immune responses. In addition, changes were observed in genes involved in extracellular matrix organisation and signalling related to cell proliferation. Changes within the same pathway, however, can be different between cell types and mechanisms underlying these differences are not known.

5.4.2 On *in vitro*-adapted cells

Two of the relevant cell types in this experiment were HUVEC and HEPG2, which are derived endothelial cells and hepatocytes, and have been adapted into *in vitro* environment. The extent to which HUVEC and HEPG2 cells represent true (*in vivo*) endothelial cells and hepatocytes was evaluated. HUVEC (in Basch media) expressed most of the genes of endothelial cell surface marker (over 90%). The high percentage may not be surprising because HUVEC is not a transformed cell line; therefore, the cells might not have adapted to a prolonged *in vitro* environment. Arguably, the differences between HUVEC and true endothelial cells may be in the gene expression levels.

For HEPG2, the cell line has been used by others for studying immune responses and responses to liver pathogen (Conceição *et al*., 2008; Israelow *et al*., 2014; Shavva *et al*., 2013) and most of the complement component were expressed in HEPG2 (C1q and C9 missing) (Morris *et al*., 1982). Some biological processes are clearly less complete in HEPG2 cells than in native liver cells (Tyakht *et al*., 2014) and it might therefore not be possible to draw a full picture of hepatocyte responses to schistosomules (or any treatment *in vitro*) when using HEPG2 as a model. However, some parts of important processes were transcriptionally active and presumably functioning and therefore can be useful to provide insights into real hepatocyte responses.

GripTite was included in the experiment to represent irrelevant cells where *S. mansoni* are unlikely to encounter in the infections. Although it is known as a modification of human embryonic kidney cells, it should not be considered as an *in vitro* counterpart of kidney epithelial or fibroblast. Instead it appears to have neuronal origin and possibly came from adrenal gland which locates close to kidney

(Stepanenko and Dmitrenko, 2015). Despite being irrelevant to *S. mansoni* infection, the cells were affected by the presence of the parasites and appeared to induce activation of immune responses and multiple signalling processes. This is likely to be generic innate responses to the parasites, but other irrelevant cell types may respond differently.

5.4.3 On aged media

In addition to the *in vitro* nature of the cells, their functions may be affected by media used in the experiment. The procedure transferred half of the old media to a new plate, meaning that overall media became older over time (as mentioned previously in chapter 4). In contrast, the worm-free cells were grown in fresh media for four days. Ideally, there should be a conditioned media control where worm-free cells received media that have been transferred over the same timecourse but without the presence of schistosomules. Alternatively, the media could be completely changed during the schistosomule transfer, but this would pose a risk of parasite loss or damage. The aged media may affect the cells, hence, it is important to bear in mind that the effect observed between the co-cultured and worm-free cells could be caused by the parasites, as well as by aged media. However, Basch media used in the co-culture was very rich in nutrient and still supported growth of co-cultured cells in the later time points. PCA also shows that time had little effect of transcriptomic profiles compared to the co-culture/worm-free factor. It is therefore not possible to entirely rule out the effect of aged media in this experiment, but it is unlikely to have been a major confounder.

5.4.4 Biological functions affected by parasite co-culture

5.4.4.1 Endothelial-leukocyte adhesion molecules

HUVEC co-cultured with schistosomules exhibited down-regulation in genes encoding endothelial-leukocyte adhesion molecules. Previous studies investigated three adhesion molecules (VCAM1, ICAM1, and SELE) and reported two of the adhesion molecules VCAM1 and SELE, being down-regulated in human lung endothelial cells treated with lipophilic portion ES from lung stage schistosomules (Trottein *et al*., 1999b). While our data is consistent regarding the down-regulation of *VCAM1* and *SELE*, I also found *ICAM1* being down-regulated. Multiple factors could contribute to the additional down-regulated gene, including the use of whole

schistosomules instead of ES portion, and different sources of endothelial cells. Expression of SELE, ICAM1, and VCAM1 on endothelial cells are involved in recruitment of leukocytes to the site of infection. SELE and ICAM1 in particular are relevant for recruitment of neutrophil (Carlos and Harlan, 1994; Lomakina and Waugh, 2009) which is capable of killing schistosomules (Incani and McLaren, 1981). For eosinophil, another effector cell that could mediate killing of schistosomules (Capron *et al*., 1979), the tethering is more dependent on selectin-P (SELP) than SELE (Kitayama *et al*., 1997) but SELP was not affected by the co-culture. The down-regulation of these adhesion molecules suggest an anti-inflammatory phenotype of co-cultured HUVEC.

5.4.4.2 Antimicrobial peptide

Similar to the observations in HUVEC, co-cultured HEPG2 cells also showed an anti-inflammatory responses. In particular, four genes with massive down-regulation encodes proteins which can function as antimicrobial peptides. The effects of antimicrobial peptides on schistosomes are not well known but they may have inhibitory roles by inducing inflammation around the parasites or by directly weakening the parasites. For example, an invertebrate antimicrobial peptide (dermaseptin) is lethal to *S. mansoni* adults *in vitro* and it also leads to reduced egg production and tegumental damage (de Moraes et al., 2011). Using antimicrobial peptide as an alternative treatment for schistosome infections has previously been proposed (Oyinloye *et al*., 2014). The four peptides down-regulated in the present study would clearly be good starting points for such a study.

5.4.4.3 Complement

In addition to cell-mediated immune responses, *S. mansoni* also needs to evade damage from complement cascade activation. Multiple strategies are known to be in place such as tegument receptor for complement components, acquiring inhibitor from host cells, and degradation of complement components (Da'dara *et al*., 2016a; Schroeder *et al*., 2009; Skelly, 2004). Our data show that *S. mansoni* may also modulate host complement production in HEPG2. Complement component genes down-regulated were *CFB, C1R, C1S, C4B, C5, C8B,* and *C8G* encompassing classical and alternative pathway, and formation of membrane attack complex. Interestingly, C3 was not affected at transcript level despite its importance at the start

of the alternative pathway and as a hub where all complement pathways converge. In comparison, C5, which also connects the three complement pathways, were down-regulated. Indeed, *S. mansoni* seem to have other strategies for regulating C3, such as receptor binding, C3 degradation, and tegument turn-over (Da'dara *et al*., 2016a; Schroeder *et al*., 2009). Additionally, C3 may be required in the hosts for other processes including developmental control, and liver regeneration (Hess and Kemper, 2016; Inal, 2004), implying that any response that *S. mansoni* provokes, needs to balance parasite and host survival.

5.4.4.4 Coagulation

Residing in blood vessels, *S. mansoni* would be expected to induce coagulation through interrupted blood flow and through damage induced from anchoring and migration. However, modulating coagulation may be important for *S. mansoni* infection due to the cross-talk between coagulation and innate immune response pathways; components in each of the two pathways can stimulate activation of the other pathway (Esmon *et al*., 2011; Markiewski *et al*., 2007), and enabling easy migration in the bloodstream. Multiple parasite-derived molecules can modulate the process (Mebius *et al*., 2013; Ranasinghe *et al*., 2015b). Here, our data show that *S. mansoni* might also regulate expression of components in coagulation pathways at their site of production (hepatocytes). The component affected in co-cultured HEPG2 ranged from those that initiate the coagulation cascade, formation of blood clots, and degradation of formed blood clots. Amongst these, down-regulation of three different fibrinogen chains – key components of blood clots – strongly suggests an anti-coagulation profile. Inhibitors of plasmin, an enzyme that degrades blood clots, were down-regulated in all three co-cultured cell types. This may explain a previous study in which blood from infected mice is more readily degraded (Da'dara *et al*., 2016b), but the effect of older parasites (liver stages and adults) on hepatocyte gene expression would need further investigation.

5.4.4.5 Tissue repair, signalling, cell cycle, and extracellular matrix reorganisation

Some genes responded differently between co-cultured cell types and the responses reflect physiological roles of the genes in that tissues. Examples of this are the down-regulation of *ICAM1* and *BMP4* in co-cultured HUVEC, but their up-regulation in co-

cultured HEPG2, with both genes having roles in liver regeneration (Oumi *et al*., 2012; Selzner *et al*., 2003). In addition, complement component *C3*, as mentioned previously, was not down-regulated in co-cultured HEPG2 and the expression level may be maintained for liver regeneration. Mechanisms that govern these differences are unknown but this differences between cell types may infer specific responses between parasites and different host tissues.

During infections with schistosomes, host tissues such as lung endothelial cells and liver hepatocytes are damaged (Bloch, 1980; Crabtree and Wilson, 1986; Torre-escudero *et al*., 2014). In this experiment, however, it is unlikely that the co-cultured cells were damaged by the parasites (and require tissue regeneration), because the parasites remained small and their oral and ventral suckers were not well developed. Although microscopic damages *in vitro* cannot be ruled out, the responses of liver-regenerating genes in HEPG2 may suggest that the presence of parasites could prompt the host cells to prepare for potential damage. Alternatively, the expression of genes related to liver regeneration may reflect HEPG2 origin of carcinoma cells. Another example of a preparation for tissue repair may come from the changes in co-cultured HUVEC. Notch signalling was one of the pathways up-regulated in co-cultured HUVEC. For endothelial cells, Notch signalling is important for homeostasis and development of blood vessel (Hofmann and Iruela-Arispe, 2007), regulating cell cycle (Noseda *et al*., 2004), and wound healing (Chigurupati *et al*., 2007). Furthermore, cell cycle genes were clearly affected in co-cultured HUVEC, and ECM organisation was affected by the production of collagen and proteases that reorganise the ECM.

The changes in these processes, however, could have other functions apart from preparation for tissue repair. For example, interference with host ECM is thought to be important for stages that invade host tissues, such as passing of eggs, miracidia, and sporocysts (Yoshino *et al*., 2014). In addition, schistosomules also degrade ECM (McKerrow *et al*., 1983). Such process may help with migration through capillary, or help with adhesion to host tissues. Indeed, schistosomules show preference for adhesion to endothelial cells compared to irrelevant cells (Trottein *et al*., 1999a). Moreover, in bacteria and protozoa, the pathogens modified host ECM to enhance their adhesion to the host tissues (Singh *et al*., 2012).

5.4.5 On *in vivo* validation

Given the *in vitro* nature of the experiment, cautions must be taken in interpreting its relevance *in vivo*. First, the host cells will have adapted to *in vitro* growth, with selection for rapidly growing cells; and second, additional factors *in vivo* could influence gene expression. Validation from *in vivo* system should be used to confirm the findings. However, many *in vivo* observations use rodents as an experimental model; whereas the *in vitro* experiment in this thesis used cells derived from humans. This could lead to inconsistent findings due to differences between organisms. Yu *et al*. (2010) investigated differences in livers of humans, mice, and rats and showed that there are differences in basic cellular processes (such as tricarboxylic acid cycle), cytokine-and cytokine receptor interaction, and complement and coagulation cascade (Yu *et al*., 2010). Many of these could be altered in *S. mansoni* infection; therefore, where possible, validation of effects represent in this chapter should derive from infection in human.

5.4.6 Summary

In this chapter, I investigated transcriptomic profiles of *in vitro* human cells co-cultured with schistosomules and showed that multiple biological processes related to infection of *S. mansoni* were affected, including immune responses, coagulation, alteration of host environment and signalling pathways. This information provides further understanding on how host biological processes might be modulated by the parasites. It is important to bear in mind the artificial aspects of the *in vitro* system and that follow up work should be performed, preferably from human infection, to fully understand the effect of the host-parasite interactions.