Integrative gene network analysis identifies TIMP1 as key factors for Haemophilus parasuis infection

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Abstract

Haemophilus parasuis (H.parasuis), an important swine pathogen, causes Glässer's disease leading to pulmonary fibrosis, polyserositis, meningitis, and arthritis. However, the common molecular response and reaction from the host remain unknown. In this study, to uncover novel host factors involved in H.parasuis infection, we identified the global transcriptomics of porcine lung, spleen, blood, alveolar macrophages (PAM), peripheral blood mononuclear cell (PBMC) and aortic vascular endothelial cells (PAVECs) after infection of H.parasuis (Hps0165 strains) using microarray data and high throughput sequencing from Gene Expression Omnibus (GEO), respectively. The results showed that fifteen overlapped genes were significantly regulated in H.parasuis infected porcine lung and spleen, and then were compared with the data from porcine blood, revealing RETN, TIMP1 and C4BPA play potentially an important role for H.parasuis invasion. Furthermore, through analysing porcine cells infected with H.parasuis, we uncover the only overlap gene TIMP1 remarkably upregulated in all assembled data, indicating that TIMP1 could function as key target for the treatment of H.parasuis infection.

Introduction

Haemophilus parasuis is a significant pathogen in contemporary swine production systems and cause Glässer's disease characterized by acute systemic inflammation of fibrinous polyserositis, meningitis and polyarthritis, leading the devastating losses to the pig industry[1,2]. Moreover, H.parasuis can be frequently isolated from the upper respiratory tract of healthy pigs. Therefore, once the virulence strains emerge, H.parasuis has become an increasing threat in pig herds of high health status, especially early-weaned pigs[3]. Currently, although successful vaccination has been achieved to control mortality, multiple different genotypes and serotypes of H.parasuis frequently result in poor cross-protection of vaccine[4,5]. H.parasuis infection requires adhesion to and invasion of host cells, resistance to phagocytosis by macrophages, resistance to serum complement and induction of inflammation. Host-pathogen interactions are of great importance in understanding the pathogenesis of infectious microorganisms and host factors play key role for the microorganisms' invasion, and have the potential to become novel broad-spectrum targets for antibacterial drugs[6,7]. However, there is lack of systematic comparison of responses from those diverse sorts of tissues and cells invaded with H.parasuis.

With the development of microarray and high throughput sequencing technologies, genome-wide molecular expression profiling has been adopted to identify key genes over the decades, providing the chances to identify the potential genes involved in H.parasuis infection. In this study, we aim to investigate the potential crucial genes in H.parasuis infection and invasion through an integrative bioinformatic analysis of gene expression profiling in public datasets. We identify TIMP1 upregulated consistently in the diverse sorts with H.parasuis infection and predicted that TIMP1 may function pivotally in H.parasuis infection and be as a potential drug targets for anti-bacterial therapy.

Materials And Methods

Data collection and processing

We downloaded the gene expression profiles related with H.parasuis infection from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) and exploited as discovery datasets to identify DEGs (Table 1). Then we screened the differentially expressed genes (DEGs) between H.parasuis infection and controls by using the “limma” R package. Genes with |log2 fold change (FC)| larger than 1 and adj P-value < 0.01 were statistically considered as the cut-off criterion. At the same time, genes with multiple probes were collapsed to keep probes with the most fidelity P value within DEGs. In addition, mRNA, miRNA and LncRNA expression profiles data of PAVECs were acquired from the published papers[8,9]. Pheatmap, ggplot2 and vennDiagram packages of R were applied to generate heatmap, volcano plot and Venn diagram, respectively, for the visualization of the identified and overlapped DEGs.

Table 1
Details for GEO data

GEO	Sample types	Experiment types
GSE11787	Spleen	Microarray[10]
GSE19126	Lung	microarray[11]
GSE30172	PAM	microarray[12]
GSE34544	PAM	microarray
GSE113252	PAVECs	HTS[13]

Abbreviation: GEO, Gene Expression Omnibus. PAM, porcine alveolar macrophages. PAVECs, porcine aortic vascular endothelial cells. HTS, high throughput sequencing.

Results

Gene transcriptional profiles of porcine spleen and lung in response to H.parasuis infection

Through comparative analysis, we found that 264 transcripts showed a level of expression that differed significantly from that of the control group with H.parasuis serovar 5 SH0165 (HPS0165) strain infected group, while a total of 89 genes were identified in porcine lung infected with HPS0165 strain compared with uninfected tissues (Fig. 1A and 1B, Supplementary S1). At the same time, we integrated the DEGs of spleen and lung and found that There were 15 overlapping genes (ALAS2, SOD2, C4BPA, TCN1, CXCL2, LTF, PDK4, TGM3, TIMP1, CRABP1, NREP, RETN, DGAT2, UPP1 and CD163) between the two datasets (Fig. 1C). The volcano plot of the datasets was shown in Fig. 1D and E.

Gene transcriptional profiles of porcine alveolar macrophages in response to H.parasuis infection

Porcine alveolar macrophages (PAMs) are important lung tissue-resident professional phagocytes and play a central role in inflammation and host defense. Hence, to uncover the key genes of PAM in response

to H.parasuis, we collected two different gene expression profiles of GSE30172 and GSE34544 infected by HPS5 and HPS4, respectively. The results showed that the screening of GSE30172 identified 257 DEGs, 204 genes which were up-regulated and 53which were down-regulated (Fig. 2A). Similarly, we obtained DEGs from GSE34544, only 20 genes which were up-regulated and 3 which were down-regulated (Fig. 2B). 11 overlapping genes (C1H15orf48, RNF128, TIMP1, CXCR6, CXCL14, IGHA, IGG2B, S100A4, GBP1, CALHM6 and LTB) were found between these two datasets (Fig. 2C). In the meantime, the overlap genes were shown using the volcano plot in Fig. 2D and E.

TIMP1 may be a key gene for H.parasuis infection

Comparing with the four datasets above, we found that TIMP1 was the only overlapping gene and significantly upregulated by H.parasuis in porcine diverse tissues or cells, indicating that TIMP1 could play an important role during H.parasuis invasion. Further, to verify this conjecture, we analyzed TIMP1 mRNA expression in porcine blood, peripheral blood mononuclear cell (PBMC) and aortic vascular endothelial cells (PAVECs), and found that TIMP1 mRNA was significantly overexpressed in all datasets (all P value < 0.01, Fig. 3A,3B and 3C), suggesting that TIMP1 may play an important role in H.parasuis infection.

Discussion

Haemophilus parasuis infection is a constant threat to the swine industry and lead to severe acute systemic infection, characterized by fibrinous polyserositis, polyarthritis and meningitis. Therefore, early assessment and treatment are essential to control infection rate and the mortality. To identify the key factors specially involved in H.parasuis infection, Our study was designed to take advantages of the large collection of transcriptome data of different hosts infected with H.parasuis based GEO database. In our study, we found that H.parasuis infection could produce a mass of differential expressed genes in different hosts, but the ovlapped genes among different hosts were rarely thought integrated analyses. TIMP1 was only uncovered to act as a potential key gene associated with the invasion of H.parasuis.

TIMP1 is a glycoprotein belonging to the member family of Tissue inhibitor of metalloproteinase and is an endogenous inhibitor of matrix metalloproteinases (MMPs)[14] to regulates the extracellular matrix (ECM) turnover and remodelling during normal development and pathogenesis. The virulence-associated trimeric autotransporters (VtaAs) in H.parasuis was found containing collagen domains and binding to extracellular matrix proteins for adherence to the host[15]. In addition, TIMP1 has been identified to be beneficial for vascular integrity and can interact with CD63/integrin β 1 complex and regulate FAK/RhoA signaling to protect blood-brain barrier function[16]. The virulence-associated trimeric autotransporters (VtaAs) in H.parasuis was found containing collagen domains and binding to extracellular matrix proteins for adherence to the host[17]. Moreover, TIMP1 also plays an important role of anti-inflammatory and antinociceptive[18], indicating that TIMP1 may be a critical component of a signalling cascade involved in reducing inflammatory hypersensitivity. H.parasuis infection activated the inflammatory signaling molecules and produced several pro-inflammatory cytokines in porcine cells[19,20]. Additionally,

highly virulent H. parasuis infection increased a proportion of CD163 + monocytes in pigs, which are able to produce high amounts of proinflammatory cytokines, such as TNF-α, IL-1 and IL-6[21]. Furthermore, Studies have shown that the expression of TIMP1 can be statistically significantly regulated by TGF-β1[22]. TGF-β1 plays an important role during the invasion of H.parasuis into cells by regulating the expression of extracellular matrix proteins[23]. We summarize the above and found that TIMP1 may be involved in the pathogenic mechanism of pathogens.

Prior works indeed showed that TIMP1 regulated pathogens infection. After P. aeruginosa infection, adequate endogenous expression of TIMP-1 in cornea protects against basement membrane and extensive corneal tissue destruction[24,25].

However, the role of TIMP1 in virus infection may act as a contrary result compared to bacteria. Coxsackievirus B3 (CVB3) infection induced a higher level of TIMP1 mRNA expression, TIMP1 knockout mice exhibited an increased survival and attenuation of myocarditis as well as reducing viral replication[26]. TIMP1 knockout mice showed considerably decreased inflammatory infiltrates in lungs compared to wild type after influenza virus infection[27]. Although the differences role of TIMP1 between virus and bacterial so far is unclear and need to be further uncovered to understand the molecular characteristics, TIMP1 may act as key roles for regulating pathogen infection.

In conclusion, we analysis and compared gene expression profile of porcine different tissues or cells in response to H.parasuis infection through transcriptional analysis based GEO database. Our data uncovered H.parasuis infection could regulate abundant genes expression in diverse tissues or cells and TIMP1 was the only overlap gene upregulated with H.parasuis infection, indicating TIMP1 may play a significant role and serve as candidate targets for treatment of H.parasuis infection.

Declarations

Authors' contributions

A. Zhou designed this project and analysed and interpreted data, and contributed to the writing of the manuscript; X. Dong and HB. Chen collected the data and modified the manuscript; MY. Liu drawn the shapes; A. Zhou supervised financial support.

Acknowledgments

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Declaration of Competing Interest

The authors declare to have no conflict of interests.

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Supplementary Data

Supplementary S1 not provided with this version

Figures

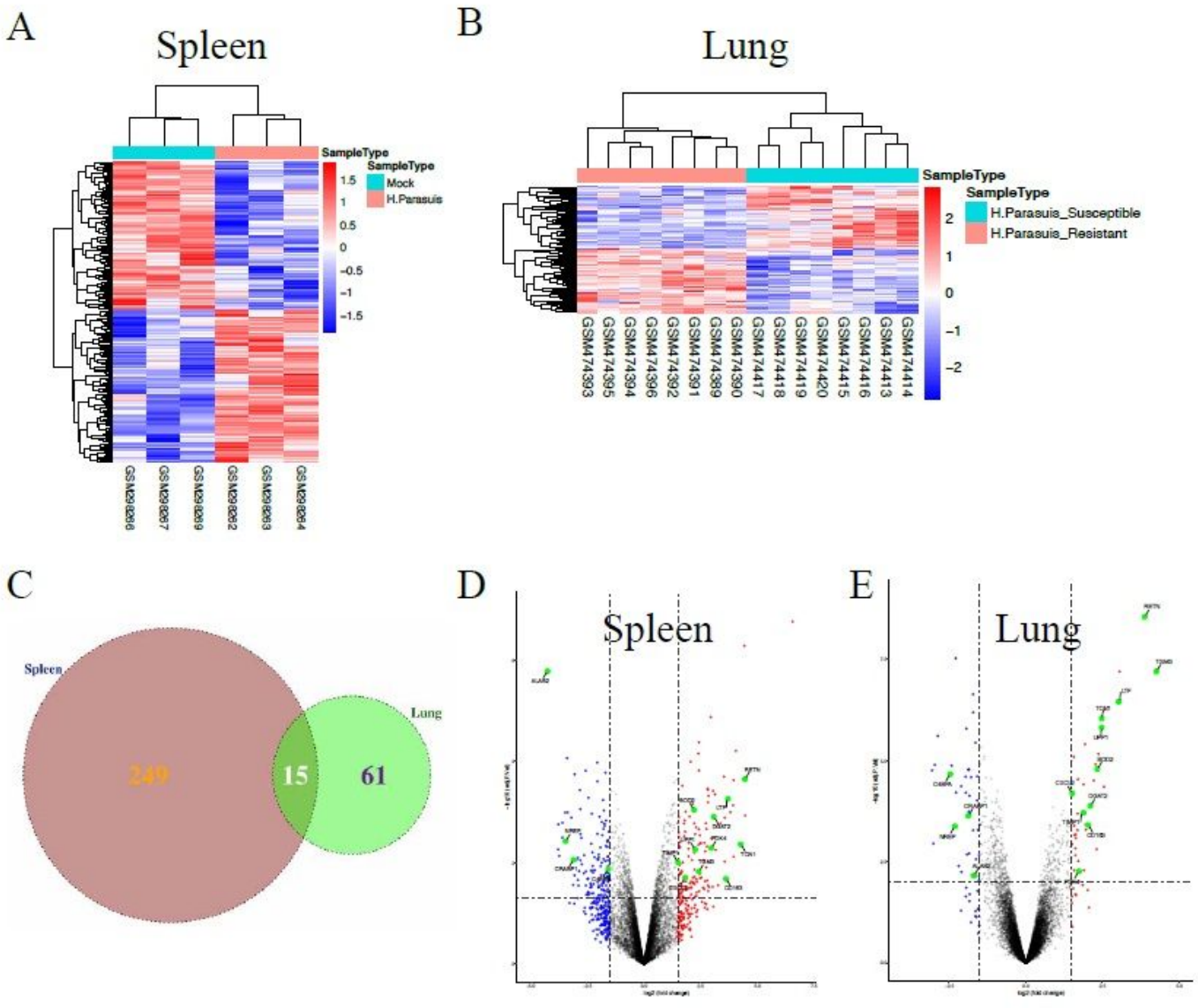

Figure 1

gene expression profiling in porcine spleen and lung tissues infected by H.parasuis infection. (A) Heatmap of differentially expression pattern of the genes in H.parasuis-infected spleen. Row represents the genes and each column corresponds to a sample. (B) Heatmap of differentially expression pattern of th genes in H.parasuis-infected lung. Row represents the genes and each column corresponds to a sample. (C) Venn diagram of the overlaps in H.parasuis-infected spleen and lung. (D) Volcano plots of the overlaps between Mock and H.parasuis-infected spleen. The vertical dashed lines correspond to 1.5-fold up and down and the horizontal dashed line represents a P value of 0.01. (E) Volcano plots of the overlaps between Mock and H.parasuis-infected lung. The vertical dashed lines correspond to 1.5-fold up and down and the horizontal dashed line represents a P value of 0.01.

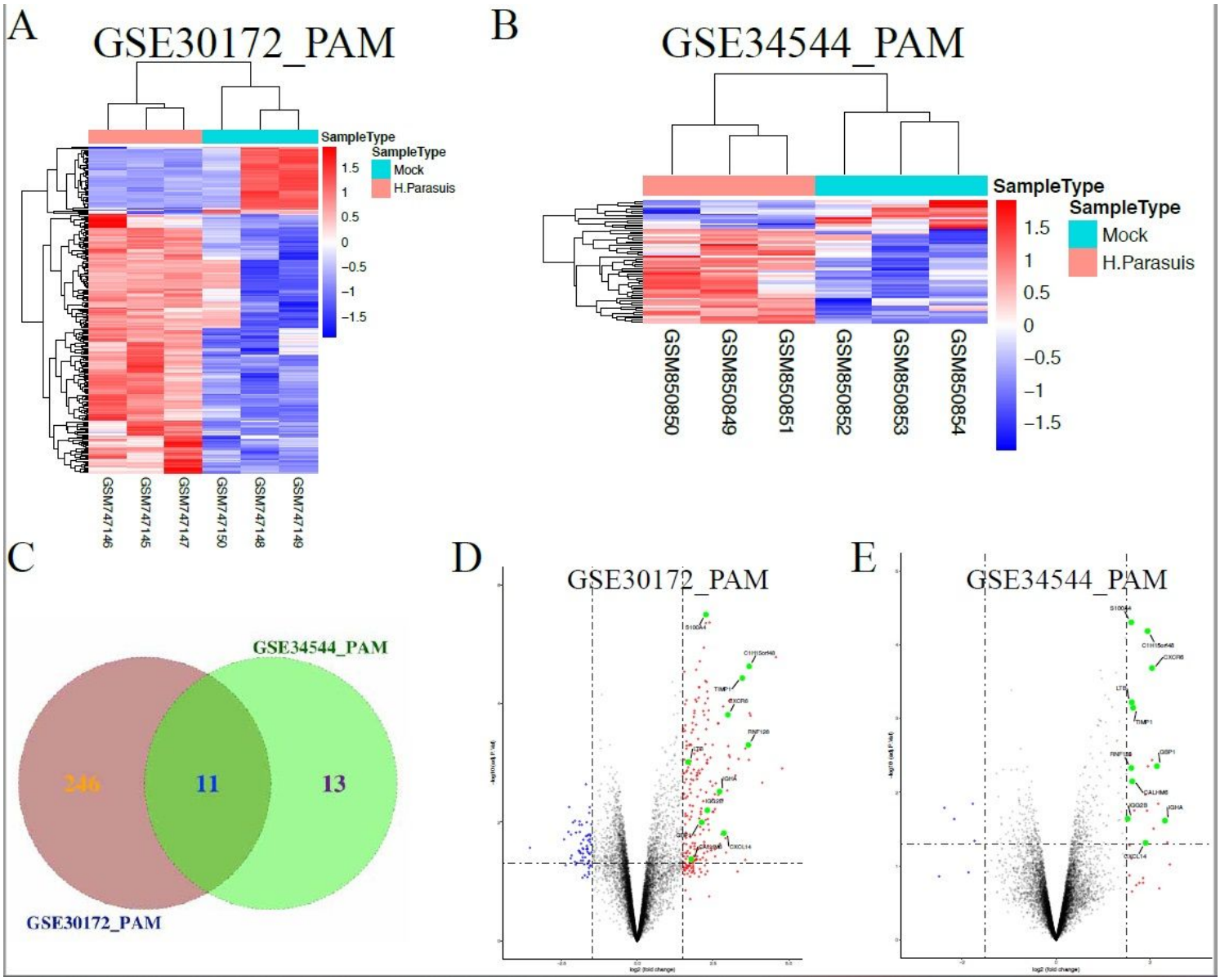

Figure 2

gene expression profiling in porcine alveolar macrophages (PAM) infected by H.parasuis infection. (A) Heatmap of differentially expression pattern of the genes in H.parasuis-infected PAM (GSE30172). Row represents the genes and each column corresponds to a sample. (B) Heatmap of differentially expression

pattern of the genes in H.parasuis-infected PAM (GSE34544). Row represents the genes and each column corresponds to a sample. (C) Venn diagram of the overlaps in H.parasuis-infected PAM. (D) Volcano plots of the overlaps between Mock and H.parasuis-infected PAM (GSE30172). The vertical dashed lines correspond to 1.5-fold up and down and the horizontal dashed line represents a P value of 0.01. (E) Volcano plots of the overlaps between Mock and H.parasuis-infected PAM (GSE34544). The vertical dashed lines correspond to 1.5-fold up and down and the horizontal dashed line represents a P value of 0.01.

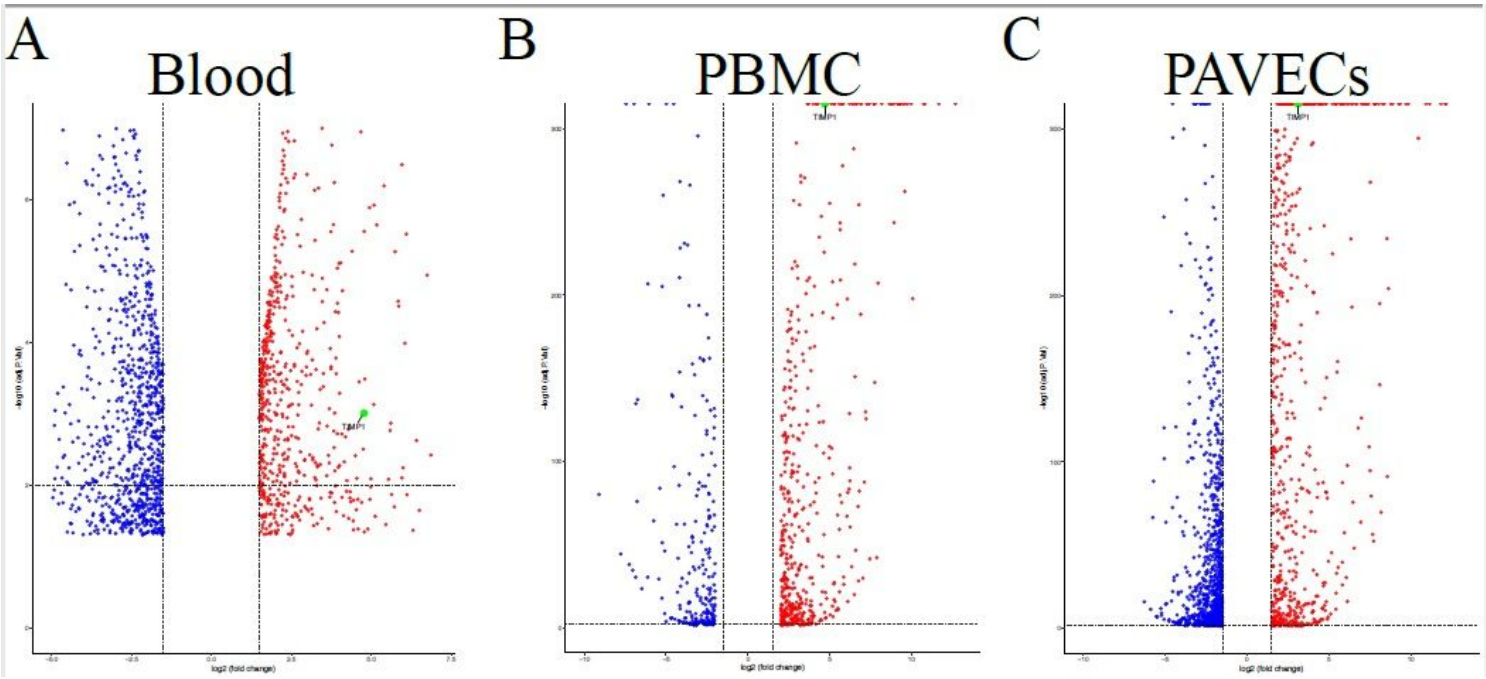

Figure 3

TIMP1 wad upregulated in differ type of samples by H.parasuis infection though next generation sequecing. (A) Volcano plots presents TIMP1 expression in bloods samples infected with H.parasuis. The vertical dashed lines correspond to 1.5-fold up and down and the horizontal dashed line represents a P value of 0.01. (B) Volcano plots presents TIMP1 expression in PBMC samples infected with H.parasuis. The vertical dashed lines correspond to 1.5-fold up and down and the horizontal dashed line represents a P value of 0.01. (C) Volcano plots presents TIMP1 expression in PAVEC cells infected with H.parasuis. The vertical dashed lines correspond to 1.5-fold up and down and the horizontal dashed line represents a P value of 0.01.