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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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. 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.

| 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 53 which 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, IGH, 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 overlapped 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) 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. 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. 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. Moreover, TIMP1 also plays an important role of anti-inflammatory and antinoiceptive, 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. 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-621. Furthermore,
Studies have shown that the expression of TIMP1 can be statistically significantly regulated by TGF-β122.
TGF-β1 plays an important role during the invasion of H.parasuis into cells by regulating the expression
of extracellular matrix proteins23. 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 destruction24,25.

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
replication26. TIMP1 knockout mice showed considerably decreased inflammatory infiltrates in lungs
compared to wild type after influenza virus infection27. 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.

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Declaration of Competing Interest

The authors declare to have no conflict of interests.

References
1. Hong-Bo Ni, Qing-Long Gong, Quan Zhao, Xiao-Yue Li, Xiao-Xuan Zhang (2020). Prevalence of Haemophilus parasuis"Glaesserella parasuis" in pigs in China: A systematic review and meta-analysis. Prev Vet Med 182:105083.

2. Kirsten C Eberle, Samantha J Hau, Shi-Lu Luan, Lucy A Weinert, Judith A Stasko, Jinhong Wang, Sarah E Peters, Paul R Langford, Andrew N Rycroft, Brendan W Wren, Duncan J Maskell, Alexander W Tucker, Susan L Brockmeier (2020). Generation and Evaluation of a Glaesserella (Haemophilus) parasuis Capsular Mutant. Infect Immun 88:e00879-19.

3. Elena Melnikow, Saffron Doman, Carole Sargent, Michael Duszenko, Gary Evans, Nikolas Gunkel, Paul M Selzer, Heinz J Ullrich (2005). Microarray analysis of Haemophilus parasuis gene expression under in vitro growth conditions mimicking the in vivo environment. Vet Microbiol 110:255-63.

4. Huisheng Liu, Qiao Xue, Qiaoying Zeng, Zhanqin Zhao (2016). Haemophilus parasuis vaccines. Vet Immunol Immunopathol 180:53-58.

5. Mar Costa-Hurtado, Emili Barba-Vidal, Jaime Maldonado, Virginia Aragon(2020). Update on Glässer’s disease: How to control the disease under restrictive use of antimicrobials. Vet Microbiol 242:108595.

6. Rongrong He, Kexin Hua, Sihua Zhang, Yun Wan, Huimin Gong, Bin Ma, Rui Luo, Rui Zhou, Hui Jin (2020). COX-2 mediated crosstalk between Wnt/β-catenin and the NF-κB signaling pathway during inflammatory responses induced by Haemophilus parasuis in PK-15 and NPTr cells. Dev Comp Immunol 105:103588.

7. Kexin Hua, Huimin Gong, Qingrong Xu, Tingting Li, Bin Ma, Yangjie Li, Rongrong He, Dingren Bi, Rui Zhou, Rui Luo, Ling Zhao, Hui Jin (2020). P38 mitogen-activated protein kinase promotes Wnt/β-catenin signaling by impeding Dickkopf-1 expression during Haemophilus parasuis infection. Cytokine 136:155287.

8. Ling Guo, Jun Liu, Yunfei Zhang, Shulin Fu, Yinsheng Qiu, Chun Ye, Yu Liu, Zhongyuan Wu, Yongqing Hou, Chien-An Andy Hu (2020). The Effect of Baicalin on the Expression Profiles of Long Non-Coding RNAs and mRNAs in Porcine Aortic Vascular Endothelial Cells Infected with Haemophilus parasuis. DNA Cell Biol 39:801-815.

9. Shulin Fu, Jun Liu, Jianfeng Xu, Sanling Zuo, Yunfei Zhang, Ling Guo, Yinsheng Qiu, Chun Ye, Yu Liu, Zhongyuan Wu, Yongqing Hou, Chien-An Andy Hu (2020). The effect of baicalin on microRNA expression profiles in porcine aortic vascular endothelial cells infected by Haemophilus parasuis. Mol Cell Biochem 472:45-56.

10. Hongbo Chen, Changchun Li, Mingdi Fang, Mengjin Zhu, Xinyun Li, Rui Zhou, Kui Li, Shuhong Zhao (2009). Understanding Haemophilus parasuis infection in porcine spleen through a transcriptomics approach. BMC Genomics 10:64.

11. Jamie M Wilkinson, Carole A Sargent, Lucina Galina-Pantoja, Alexander W Tucker (2010). Gene expression profiling in the lungs of pigs with different susceptibilities to Glässer’s disease. BMC Genomics 11:455.
12. Yang Wang, Chong Liu, Ying Fang, Xiaoli Liu, Wentao Li, Shuqing Liu, Yingyu Liu, Yuxi Liu, Catherine Charreyre, Jean-Christophe Audonnet, Pin Chen, Qigai He (2012). Transcription analysis on response of porcine alveolar macrophages to Haemophilus parasuis. BMC Genomics 13:68.

13. Shulin Fu, Jing Guo, Ruizhi Li, Yinsheng Qiu, Chun Ye, Yu Liu, Zhongyuan Wu, Ling Guo, Yongqing Hou, Chien-An Andy Hu (2018). Transcriptional Profiling of Host Cell Responses to Virulent Haemophilus parasuis: New Insights into Pathogenesis. Int J Mol Sci 19:1320.

14. Brew K, Nagase H (2010). The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. Biochim Biophys Acta 1803:55-71.

15. Mar Costa-Hurtado, Laura Garcia-Rodriguez, Sergi Lopez-Serrano, Virginia Aragon (2019). Haemophilus parasuis VtaA2 is involved in adhesion to extracellular proteins. Vet Res 50:69.

16. Jingshu Tang, Yuying Kang, Longjian Huang, Lei Wu, Ying Peng (2020). TIMP1 preserves the blood-brain barrier through interacting with CD63/integrin β 1 complex and regulating downstream FAK/RhoA signalling. Acta Pharm Sin B 10:987-1003.

17. Mar Costa-Hurtado, Laura Garcia-Rodriguez, Sergi Lopez-Serrano, Virginia Aragon (2019). Haemophilus parasuis VtaA2 is involved in adhesion to extracellular proteins. Vet Res 50:69.

18. Nuttall R. K, Silva C, Hader W, Bar-Or A, Patel K. D, Edwards D. R (2007). Metalloproteinases are enriched in microglia compared with leukocytes and they regulate cytokine levels in activated microglia. Glia 55 516–526.

19. Shulin Fu, Wenhua Zhao, Chunhong Xiong, Ling Guo, Jing Guo, Yinsheng Qiu, Chien-An Andy Hu, Chun Ye, Yu Liu, Zhongyuan Wu, Yongqing Hou (2019). Baicalin modulates apoptosis via RAGE, MAPK, and AP-1 in vascular endothelial cells during Haemophilus parasuis invasion. Innate Immun 25:420-432.

20. Bouchet B, Vanier G, Jacques M, Auger E, Gottschalk M (2009). Studies on the interactions of Haemophilus parasuis with porcine epithelial tracheal cells: limited role of LOS in apoptosis and pro-inflammatory cytokine release. Microb Pathog 46:108-13.

21. Frandoloso R, Martínez-Martínez S, Yubero S, Rodríguez-Ferri EF, Gutiérrez-Martín CB (2012). New insights in cellular immune response in colostrum-deprived pigs after immunization with subunit and commercial vaccines against Glässer's disease. Cell Immunol 277:74-82.

22. Pedro Serralheiro, Elisa Cairrão, Cláudio J Maia, Marina João, Carlos M Costa Almeida, Ignacio Verde (2016). Effect of TGF-beta1 on MMP/TIMP and TGF-beta1 receptors in great saphenous veins and its significance on chronic venous insufficiency. Phlebology 32:334-341.

23. Yufeng Li, Yaning Zhang, Yuting Xia, Yijuan Shen, Jiansong Zhang. Haemophilus parasuis modulates cellular invasion via TGF-β1 signaling (2016). Vet Microbiol 196:18-22.

24. K A Kernacki, R Barrett, L D Hazlett (1999). Evidence for TIMP-1 protection against P. aeruginosa-induced corneal ulceration and perforation. Invest Ophthamol Vis Sci 40:3168-76.

25. Karen A Kernacki 1, John L Chunta, Ronald P Barrett, Linda D Hazlett (2004). TIMP-1 role in protection against Pseudomonas aeruginosa-induced corneal destruction. Exp Eye Res 78:1155-62.
26. Crocker SJ, Frausto RF, Whitmire JK, Benning N, Milner R, Whitton JL (2007). Amelioration of coxsackievirus B3-mediated myocarditis by inhibition of tissue inhibitors of matrix metalloproteinase-1. Am J Pathol 171:1762-73.

27. Jenieke R Allen, Lingyin Ge, Ying Huang, Rena Brauer, Tanyalak Parimon, Suzanne L Cassel, Fayyaz S Sutterwala, Peter Chen (2018). TIMP-1 Promotes the Immune Response in Influenza-Induced Acute Lung Injury. Lung 196:737-743.

**Supplementary Data**

Supplementary S1 not provided with this version