ORIGINAL RESEARCH

The Role of Decorin in the Adhesion Process of *Treponema Pallidum* Subspecies *Pallidum* to Human Brain Microvascular Endothelial Cells

Fan Wu, MD; Xiaoli Dai, BM; Tingyu Xue, BM; Fangzhi Du, MM; Qianqiu Wang, MD; Bin Chen, MD

ABSTRACT

Objective • This study aims to investigate the role of decorin in the adhesion process of *Treponema pallidum* subspecies *pallidum* (*T. pallidum*) to human brain microvascular endothelial cells.

Methods • The study involved an *in vitro* experimental design. Western blot analysis was conducted to determine the protein expression level of decorin in the cells. The cells were divided into four groups: Tp group, inactivated Tp group, LPS group, and negative control group. The adhesion of *T. pallidum* to the cells was analyzed using darkfield microscopy counting and quantitative polymerase chain reaction (qPCR). The cells were divided into four groups based on different preprocessing treatments: control group, decorin group, *DCN*-siRNA group, and *DCN*-siRNA+decorin group. Changes in the F-actin of the cells were explored using confocal laser scanning microscopy. The cells were divided into the Tp group, Tp+decorin group, and control group.

Results • Western blot analysis showed high expression of decorin in the Tp group and LPS group. Darkfield microscopy counting revealed a significantly higher number of *T. pallidum* adhered to a single cell in the decorin group compared to the control group. Conversely, the number of adhered *T. pallidum* was significantly lower in the *DCN*-siRNA group compared to the control group. qPCR results indicated a considerably higher *T. pallidum* load in the decorin group compared to the control group. In the Tp group, *T. pallidum* treatment induced the reorganization of F-actin, while the distribution of F-actin in the Tp+decorin group was comparable to that of the control group.

Conclusions • Decorin enhances the adhesion of *T. pallidum* to human brain microvascular endothelial cells, suggesting that decorin may act as one of the receptors regulating the adhesion of *T. pallidum* to cells. Furthermore, *T. pallidum* treatment triggers the rearrangement of F-actin in cells, and decorin plays a protective role in this process. (*Altern Ther Health Med.* 2023;29(7):68-73).

Fan Wu, MD; Xiaoli Dai, BM; Bin Chen, MD; Department of Dermatology, Sir Run Run Hospital, Nanjing Medical University, Nanjing, Jiangsu, China. **Tingyu Xue, BM,** Kangda College of Nanjing Medical University, Lianyungang, Jiangsu, China. **Fangzhi Du, MM; Qianqiu Wang, MD;** Institute of Dermatology, Chinese Academy of Medical Sciences and Peking Union Medical College, Nanjing, Jiangsu, China.

Corresponding author: Qianqiu Wang, MD
E-mail: doctorwqq@163.com
Corresponding author: Bin Chen, MD
E-mail: chenbin@njmu.edu.cn

INTRODUCTION

It has been reported that *Treponema pallidum* subspecies *pallidum* (Tp) can directly attach to human brain microvascular endothelial cells (HBMECs) *in vitro*.[1] Wu et al.[2] observed an upregulation of the gene encoding decorin in HBMECs following Tp adhesion by using high-throughput sequencing technology. Decorin is a small molecular weight dermatan sulfate proteoglycan primarily found in the extracellular matrix of tissues rich in type I collagen, such as skin, joints, and endothelial cells.[3] Previous studies have demonstrated that decorin acts as one of the critical receptors mediating the adhesion of *Borrelia burgdorferi* (*B. burgdorferi*) to host cells, thereby contributing to the pathogenicity of *B. burgdorferi*.[4,5]

However, the role of decorin in the adhesion of Tp to host cells remains unclear. Therefore, in this study, we aimed to investigate the impact of different states of Tp on the protein expression level of decorin in cells using Western blot analysis. The primary objective of this study is to elucidate the role of decorin in the process of Tp adhesion to host cells and provide novel insights into the underlying mechanisms of neurosyphilis pathogenesis.

MATERIALS AND METHODS

Study Design

The study employed a multi-faceted approach to investigate the adhesion process of Tp to human brain microvascular endothelial cells. The study design incorporated various techniques to evaluate Tp adhesion and its impact on cellular structures comprehensively. Darkfield microscopy counting was utilized to directly observe and quantify Tp adhesion to the cells, providing insights into the extent of attachment. Quantitative polymerase chain reaction (qPCR) was employed to molecularly assess Tp load in the cells, allowing for a precise measurement of adhesion at the genetic level. Additionally, immunofluorescence staining was performed to examine the effect of Tp adhesion on the cytoskeletal protein F-actin, providing insights into potential alterations in cellular structure. Ethical approval for this study was obtained from the Institutional Animal Care and Use Committee (IACUC) of the Institute of Dermatology, Chinese Academy of Medical Sciences, and Peking Union Medical College (Permit Number: 2019-KY011).

Animal Housing and Strain Used

Mature male New Zealand White rabbits (n = 6) were housed in accordance with the guidelines set by the Animal Center of the Institute of Dermatology, Chinese Academy of Medical Sciences. The rabbits were monitored for testicular swelling, and once the swelling persisted for three days, indicating optimal orchitis, the rabbits were considered suitable for the study. Euthanasia was performed by intravenous injection of pentobarbital (90mg/kg). The Tp strain (Nichols strain) used in the study was generously provided by Professor Tian-ci Yang from Zhongshan Hospital, Xiamen University. Primary human brain microvascular endothelial cells (HBMECs; ACBRI 376) were obtained from Cell Systems, USA.

Materials and Reagents

Endothelial cell complete culture medium (4Z0-500-R, Cell Systems, USA), lipopolysaccharide (LPS) (32K4092, Sigma, USA), rabbit serum (prepared in-house), fetal bovine serum (11011-8611, Hangzhou Four Seasons, China), Lipofectamine™ 2000 transfection reagent (11668027, Invitrogen, USA), DNA extraction kit (51304, Qiagen, USA), fluorescent quantitative PCR assay kit (HRR060A, TaKaRa, China), BCA protein concentration assay kit (P0010-1, Biyuntian, China), anti-*DCN* antibody (BA0798, Abcom, UK), anti-GAPDH antibody (AB-P-R001, Xianzhi, China), horseradish peroxidase-conjugated goat anti-rabbit IgG (SA00001-2, Proteintech, USA), and ECL luminescent solution (WBKLS0500, Millipore, USA).

Main Instruments

The following instruments were utilized in the study: (1) Darkfield microscope (Nikon, Japan); (2) EX70 inverted phase contrast microscope (Olympus, Japan); (3) Spectrophotometer (Beckman Coulter, USA); (4) High-speed centrifuge (Thermo Scientific, USA); (5) Fluorescence quantitative PCR instrument (ABI, USA).

Cell Culture

HBMECs were inoculated in culture flasks containing endothelial cell complete culture medium and incubated at 37°C in a 5% CO_2 incubator. Cells were passaged for 3-6 generations before being collected for further experiments.

Culture and Collection

The activated Tp suspension was cultured and prepared following previously described methods [2]. The inactivated Tp suspension was subjected to heat treatment in a water bath at 56°C for 30 minutes.

DCN-siRNA Transfection in HBMECs

DCN-siRNA was synthesized by Bioengineering (Shanghai) Co., Ltd., with the sense strand sequence of 5'-AAGUCAUGCUGCCCAACUUCGGAGA-3' and the antisense strand sequence of 5'- UCUCCGAAGUUGGGC AGCAUGACUUU-3'. HBMECs were seeded in 6-well cell culture plates and incubated at 37°C with 5% CO_2. When the cells reached approximately 40% to 50% confluence, a mixture of the specific *DCN*-siRNA sequence and Lipofectamine™ 2000 was added to the cells. Concurrently, a blank control group and a negative control group (NC-siRNA) were included. The cell cultures were maintained for 6 hours, after which the old medium and mixture were removed, and fresh medium was added for further incubation at 37°C with 5% CO_2 for 48 hours. The expression of the target gene was assessed using RT-PCR and Western blot analysis.

Cell Grouping and Treatment

Grouping of Tp-Induced HBMECs Expressing Decorin. HBMECs were randomly assigned to the following groups: (1) Tp group; (2) inactivated Tp group; (3) LPS group; and (4) negative control group. Equal amounts of Tp suspension (1.6×10^7/ml), inactivated Tp suspension (1.6×10^7/ml), LPS (200μg/L), and cell culture medium were added to their respective groups. After simultaneous incubation for 4 hours, the cells were collected for further analysis.

Grouping for Tp Adhesion Assay. The cells were divided into the following groups: (1) control group (untreated HBMECs); (2) decorin group (HBMECs pretreated with decorin for 2 hours); (3) *DCN*-siRNA group (HBMECs interfered with *DCN*-siRNA); and (4) *DCN*-siRNA+decorin group (HBMECs interfered with *DCN*-siRNA and pretreated with decorin for 2 hours). The cells were seeded in 24-well plates with 14 mm diameter circular coverslips for darkfield microscopy counting. For the q-PCR assay, the cells were seeded in 6-well plates. In each group, equal amounts of freshly prepared Tp suspension (1.6×10^7/ml) were added, and all groups were cultured simultaneously for 4 hours.

Western Blot Assay for Detection of Decorin Expression Level

Total protein samples were extracted from cells using a pre-configured cell lysis solution, and the protein concentration was determined using the BCA method. Subsequently, the samples underwent SDS-PAGE electrophoresis and were transferred to a membrane. The membrane was then incubated overnight at 4°C on a shaker with decorin monoclonal antibody (1:600 dilution) and GAPDH antibody (1:200 dilution). Following this, the membrane was incubated with a secondary antibody at room temperature for 1 hour, followed by treatment with horseradish peroxidase for 1 hour. The blot images were analyzed using Image J software to determine the relative expression levels of the target proteins. The expression level of the target protein was calculated as the grayscale value of the target protein band divided by the grayscale value of the internal reference band.

Darkfield Microscopy Counting Method

After removing the supernatant and coverslip, the cells were rinsed three times with PBS. The total number of Tp adhered to 20 cells was counted using a darkfield microscope. The amount of Tp adhered to a single cell was calculated as the total number of Tp divided by 20. The data were obtained from three independent experiments.

Detection of Tp DNA Load by qPCR

Total DNA was extracted from cells in each group, and the primer sequences were designed using Primer Premier 5.0 software. The Tp-*flaA* primer sequences were as follows: (1) upstream primer: 5'-AACGCAAACGCAATGATAAA-3'; (2) downstream primer: 5'-CCAGGAGTCGAACAGGA GATAC-3'. For β-actin, the primer sequences were as follows: (1) upstream primer: 5'-CAGGCACCAGGGCGTGA TGG-3'; (2) downstream primer: 5'-CGATGCCGTGCTCGATGGGG-3'.

Reactions conditions: The qPCR reaction conditions consisted of pre-denaturation at 95°C for 10 minutes, followed by denaturation at 95°C for 15 seconds, annealing at 55°C for 20 seconds, and extension at 72°C for 20 seconds, repeated for 40 cycles. The DNA load of Tp was calculated using a standard curve. A 10-fold dilution series of linearized plasmid DNA ranging from 1×10^7 to 1×10^1 copies were used to generate the flaA standard curve, while the β-actin standard curve was constructed by serially diluting DNA from 100ng/ml to 0.0488ng/ml. Four controls were included: (1) no DNA template; (2) no Taq polymerase; (3) no primer; and (4) DNA with a known Tp load.

Immunofluorescence Assay for Examination of F-actin

To investigate changes in the cytoskeletal protein F-actin, HBMECs were seeded in poly-Lysine coated confocal dishes and randomly assigned to the following groups: (1) Tp group; (2) Tp+decorin group; and (3) control group. The Tp+decorin group samples were pretreated with a cell culture medium containing decorin (10 mg/L) for 2 hours. Cells in the Tp group and Tp+decorin groups were exposed to freshly prepared Tp suspension (1.6×10^7 /ml), while samples in the control group were cultured with an equal volume of cell culture medium. All groups were cultured simultaneously for 4 hours.

Following incubation, the cells were treated with 4% paraformaldehyde and fixed at room temperature for 30 minutes. 0.5% Triton X-100 was added for 5 minutes to permeabilize the cells, followed by rinsing. Subsequently, rhodamine-labeled ghost pen cyclopeptide solution (1:100 dilution) was added dropwise to the cells and incubated for 30 minutes. Finally, DAPI solution (1:100 dilution) was added to the cells and incubated for 5 minutes. The cells were observed under a laser confocal microscope to visualize changes in F-actin.

Statistical Analysis

Data analysis was performed using SPSS 20.0 software (IBM, Armonk, NY, USA). Group comparisons were conducted using one-way ANOVA and ANOVA of repeated measurement data. Statistical significance was set at $P < .05$.

RESULTS

DCN-siRNA Transfection

The RT-PCR results demonstrated a significant decrease ($P < .05$) in mRNA expression level in the siRNA group compared to the blank control and negative control groups, as shown in Figure 1. Furthermore, Western blot analysis revealed a significant reduction in decorin protein expression in the siRNA group compared to the blank control and negative control groups (both $P < .05$), as depicted in Figure 2. These findings indicate that siRNA interference targeting DCN is highly efficient and specific.

Protein Expression Levels of Decorin in HBMECs in Each Group

The protein expression levels of decorin were significantly higher in the Tp group and LPS group (both $P < .05$) compared to the inactivated Tp group and negative control group. Additionally, there was no significant difference in the protein expression of decorin between the Tp group and LPS group ($P > .05$), as depicted in Figure 3 and Figure 4.

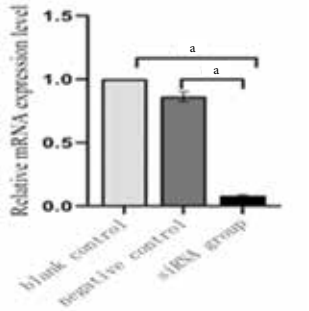
Darkfield Microscopic Observation of Tp Adherence

Under darkfield microscopy (Figure 5), a substantial number of Tp organisms were observed on each cell. The number of Tp adhered to single cells in the decorin group was significantly higher than in the control group ($P < .05$). Conversely, the number of Tp adhered to individual cells in the *DCN*-siRNA group was significantly lower compared to the control group ($P < .05$). Further analysis revealed no significant difference in the number of Tp adhered to individual cells between the *DCN*-siRNA+decorin group and the control group ($P > .05$, Figure 6).

Darkfield Microscopic Observation of Tp Adherence

Under darkfield microscopy (Figure 5), a substantial number of Tp organisms were observed on each cell. The

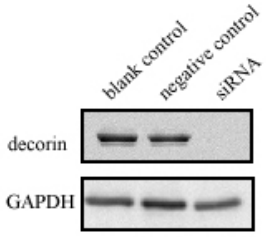
Figure 1. mRNA expression levels of decorin in HBMECs.



[a]Statistical analysis showed significant differences between the siRNA group and the blank control group, ($P < .01$).

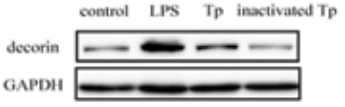
Note: The mRNA expression levels of decorin were quantified using RT-PCR. Data are presented as mean ± standard deviation (n = 3).

Figure 2. Decorin protein expression in HBMECs.



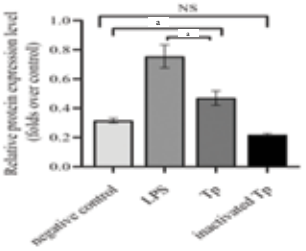
Note: Protein expression of decorin was assessed using Western blot analysis. The intensity of the decorin band was quantified and normalized to the internal control (GAPDH). Data are representative of three independent experiments. The protein expression of decorin was significantly lower in the siRNA group compared to the blank control and negative control groups ($P < .05$).

Figure 3. Protein expression of decorin in various groups of HBMECs.



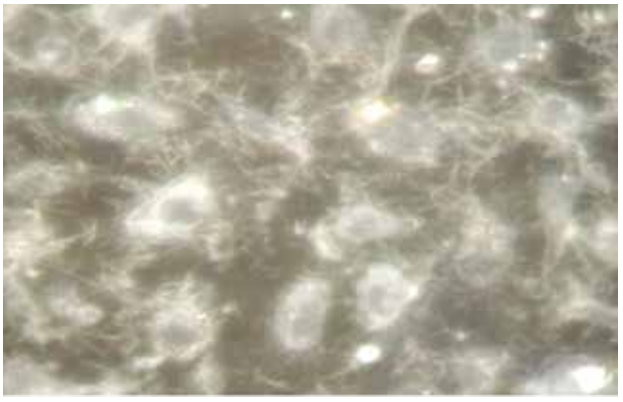
Note: HBMECs: Human brain microvascular endothelial cells; Tp: *Treponema pallidum* subspecies *pallidum*; LPS: Lipopolysaccharide. The protein expression levels of decorin were analyzed in different groups of HBMECs. Data represent the relative expression of decorin protein in each group.

Figure 4. Protein expression levels of decorin in HBMECs.



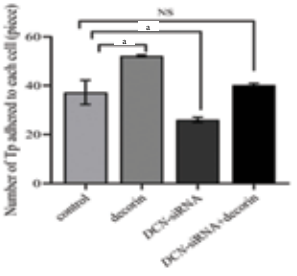
Note: HBMECs: Human brain microvascular endothelial cells; Tp: *Treponema pallidum* subspecies *pallidum*; LPS: Lipopolysaccharide.The protein expression levels of decorin were analyzed in HBMECs. Data are presented as mean ± standard deviation (n = 3). Statistical analysis revealed a significant difference in decorin protein expression between the Tp group and the control group ([a]$P < .05$). No significant difference (NS) was observed between the Tp group and the LPS group.

Figure 5. Darkfield microscopy image showing Treponema pallidum (Tp) adhered to cells (1000× magnification).



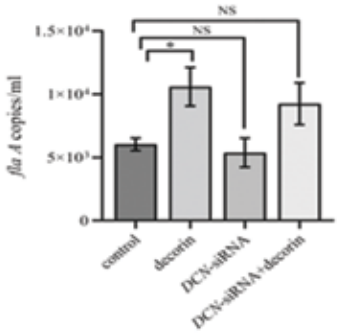
Note: The image captured under darkfield microscopy depicts the adhesion of Tp, specifically *Treponema pallidum* subspecies *pallidum*, to the cells.

Figure 6. Number of Treponema pallidum (Tp) adhered to a single cell.



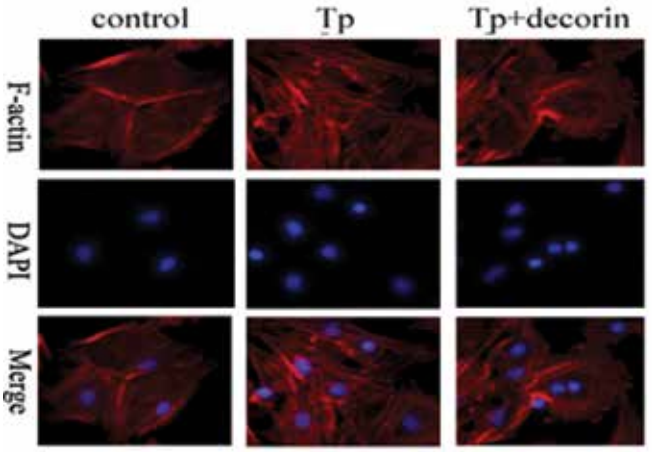
Note: Tp: Treponema pallidum subspecies pallidum. The number of Tp organisms adhered to individual cells was quantified. Data are presented as mean ± standard deviation (n = 3). Statistical analysis revealed significant differences between the decorin group and the control group ([a]$P < .05$). No significant difference (NS) was observed between the *DCN*-siRNA+decorin group and the control group.

Figure 7. Treponema pallidum (Tp) load in different groups.



Note: Tp: *Treponema pallidum* subspecies *pallidum*. The Tp load was assessed in various groups. Data are presented as mean ± standard deviation (n = 3). Statistical analysis revealed a significant difference in Tp load between the decorin group and the control group ($^{a}P < .05$). No significant difference (NS) was observed in Tp load between the *DCN*-siRNA group and the control group.

Figure 8. Changes in the cytoskeletal protein F-actin.



Note: Tp: *Treponema pallidum* subspecies *pallidum*.The figure illustrates the alterations observed in the cytoskeletal protein F-actin. The changes in F-actin distribution and organization in response to Tp adhesion are depicted.

number of Tp adhered to single cells in the decorin group was significantly higher than in the control group ($P < .05$). Conversely, the number of Tp adhered to individual cells in the *DCN*-siRNA group was significantly lower compared to the control group ($P < .05$). Further analysis revealed no significant difference in the number of Tp adhered to individual cells between the *DCN*-siRNA+decorin group and the control group ($P > .05$); see Figure 6.

DNA Load of Tp in Each Group

The Tp load in the decorin group was significantly higher than in the control group ($P < 0.05$). However, there were no significant differences in Tp load between the *DCN*-siRNA group and the *DCN*-siRNA+decorin group compared to the control group, as depicted in Figure 7.

Changes in Cytoskeletal Protein F-actin

Analysis of the cells revealed that the F-actin in the control group exhibited a disordered pattern, with thin microfilaments and the absence of fibril formation. The protein was primarily distributed around the cells. In the Tp group, F-actin levels were also increased, with stress fibers concentrated in the central region of the cells. Interestingly, in the Tp+decorin group, the distribution of F-actin closely resembled that of the control group, refer to Figure 8.

DISCUSSION

Neurosyphilis is a chronic infectious disease of the central nervous system (CNS) caused by Tp. The adhesion and traversal of the blood-brain barrier by Tp are considered crucial steps in the pathogenesis of neurosyphilis.[1,2] The blood-brain barrier comprises HBMECs as its main component, forming a physical defense barrier and immune defense barrier within the CNS to protect against pathogenic invasion. Previous research by Wu et al.[1] demonstrated the ability of Tp to adhere to the surface of HBMECs *in vitro*. Decorin, a small molecular weight dermatan sulfate proteoglycan primarily found in the extracellular matrix of type I collagen-dominated tissues, is known to modulate collagen deposition. In recent years, it has been discovered that decorin is also expressed in brain microvascular endothelial cells, where it exerts antioxidant and anti-inflammatory effects, thereby protecting neural tissue against traumatic brain injury.[6]

In this study, we observed an increase in the protein expression level of decorin in HBMECs following exposure to active Tp, suggesting that decorin may play a role in immune processes subsequent to Tp invasion into the CNS. It is important to note that although neurosyphilis is typically categorized as stage III syphilis, it can manifest at any stage of Tp infection in humans.[7] Tp is known for its high neuroinvasiveness and can be detected in the cerebrospinal fluid shortly after infection. A previous study[8] revealed that Tp was present in the cerebrospinal fluid of 24% of untreated syphilis patients and up to 40% of stage I syphilis patients. However, it is important to note that many cases of neurosyphilis exhibit mild or no clinical manifestations.

This characteristic of Tp is attributed to its robust immune evasion capability, enabling it to evade elimination by the host immune system and establish a persistent long-term infection. Studies have suggested that upregulation of IL-10 expression in the cerebrospinal fluid, resulting in a downregulation of the inflammatory response, may promote the survival of Tp in the CNS.[9] Therefore, it is worth further investigating whether the anti-inflammatory effect exerted by decorin creates a favorable environment for Tp invasion into the CNS.

Tp possesses the ability to traverse multiple cellular basement membranes before entering blood vessels, thus enabling its dissemination into other tissues such as muscles

and nerves. This process requires strong adhesion and penetration capabilities. Various membrane proteins of Tp play a role in the adhesion process. Notably, certain membrane proteins of Tp, namely Tp0483, Tp0155, and Tp0136, are involved in the adhesion to fibronectin.[10-13] Another Tp membrane protein, Tp0751, is known to adhere to laminin.[14]

Laminin, a major constituent of the cellular basement membrane, serves as an important ligand for Tp adhesion, facilitating the crossing of the basement membrane and subsequent invasion of tissues through the bloodstream [14]. In the current study, the supplemental administration of decorin was found to enhance the adhesion of Tp to HBMECs, implying that decorin may act as one of the receptors involved in mediating the adhesion of Tp to host cells. Pathogenic bacteria can invade host cells by modulating the motility and plasticity of the cytoskeletal protein F-actin, which plays a critical role in initiating cytoskeletal rearrangement. This rearrangement facilitates cell contraction and the formation of gaps that enable pathogenic microorganisms to traverse cell barriers.[15]

Haake et al.[16] demonstrated the ability of Tp to cross human umbilical venous endothelial cells. In our study, we observed the rearrangement of F-actin in HBMECs following Tp adhesion, indicating the contraction of HBMECs due to Tp adhesion. Interestingly, the changes in F-actin in the decorin group resembled those in the control group, suggesting a protective effect of decorin against Tp-induced cell contraction. However, the precise mechanisms underlying this protective effect require further investigation.

Study Limitations

There are certain limitations to be considered in this study. First, the experiments were conducted *in vitro* using HBMECs, which may not fully represent the complex physiological conditions present in the human body. Therefore, caution should be exercised when extrapolating the findings to clinical situations. Second, the study focused on the role of decorin in the adhesion process of Tp to HBMECs, but the exact molecular mechanisms involved were not elucidated. Further research is needed to explore the specific signaling pathways and interactions underlying this process.

Additionally, the study utilized a specific strain of Tp (Nichols strain), and it would be valuable to investigate whether similar effects are observed with other strains or subspecies of Tp. Finally, the sample size in this study was relatively small, and further studies with larger sample sizes are warranted to validate the findings and enhance the statistical power. Despite these limitations, our study contributes to understanding the adhesion process of Tp to host cells and provides a basis for future research in this area.

CONCLUSION

In conclusion, our study provides novel insights into the role of decorin in the adhesion process of Tp to HBMECs. We have demonstrated that active Tp enhances the protein expression of decorin in HBMECs, and this upregulation of decorin promotes the adhesion of Tp to HBMECs. These findings suggest that decorin may act as one of the receptors mediating the adhesion of Tp to host cells. Additionally, our study reveals that Tp adhesion induces rearrangement of the cytoskeletal protein F-actin in HBMECs. Collectively, these results enhance our understanding of the complex interactions between Tp and host cells, providing valuable insights into the pathogenesis of Tp-related infections.

DATA AVAILABILITY

The experimental data used to support the findings of this study are available from the corresponding author upon request.

CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest regarding this work.

AUTHORS' CONTRIBUTIONS

All authors contributed equally; they read and approved the final manuscript.

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