Identification of an activation-related protein in B cells in the ABO incompatible condition

JINGSONG CAO[1-3*], CONG CHEN[1*], LUOGEN LIU[2], YUNSHENG ZHANG[2], HONG ZHOU[3], JIANHUA XIAO[1] and YI WANG[2,4]

[1]Clinical Research Center, Institute of Pathogenic Biology, Medical College, Hunan Provincial Key Laboratory for Special Pathogens Prevention and Control, University of South China; [2]The Second Hospital, University of South China; [3]The First Affiliated Hospital of University of South China, Hengyang, Hunan 421001; [4]The Second Affiliated Hospital of Hainan Medical University, Haikou, Hainan 570102, P.R. China

Received May 24, 2019; Accepted October 22, 2019

DOI: 10.3892/etm.2019.8234

Abstract. In ABO-incompatible (ABOi) kidney transplantation (KT), antibodies can mediate immunological accommodation or immune rejection, but the mechanism by which B cells are induced to produce antibodies with different functions is still unclear. Previous research established an ABOi kidney cell model and identified that haptoglobin (HP) is associated with the activation of lymphocytes. In the present study, the results of a flow cytometric assay demonstrated that HP was expressed by B cells. Moreover, dot-ELISA and ELISA analyses showed that the concentrations of total IgG, blood group B antibody, IgG1, IgG2 and IgG4 were all significantly increased in the cell model. In addition, dot-ELISA and haptoglobin level analyses showed that HP protein expression was significantly increased, while RT-qPCR assay indicated that HP was significantly reduced at the mRNA level. Furthermore, bioinformatics analysis showed that HP could interact with Smad3, and the HP-Smad3 complex was detected in a peripheral blood mononuclear cell (PBMC) protein extract by a dot-ELISA method. This research revealed that HP was involved in the process of B-cell activation by interacting with Smad3, and the results will be helpful to reveal the mechanism of B-cell activation in ABOi-KT.

Correspondence to: Professor Yi Wang, The Second Affiliated Hospital of Hainan Medical University, Haikou, Hainan 570102, P.R. China
E-mail: wayne0108@126.com

Professor Jianhua Xiao, Clinical Research Center, Institute of Pathogenic Biology, Medical College, Hunan Provincial Key Laboratory for Special Pathogens Prevention and Control, University of South China, Hengyang, Hunan 421001, P.R. China
E-mail: jhxiao223@163.com

*Contributed equally

Key words: haptoglobin, ABOi-KT, B cells, Smad3, immunological accommodation

Introduction

Since the first ABO-incompatible (ABOi) kidney transplantation (KT), was carried out in Japan in 1989 and excellent long-term outcomes were obtained in subsequence surgeries (1-3), the number of cases of ABOi-KT have gradually increased worldwide. To avoid graft rejection in ABOi-KT, immunosuppression is usually used to maintain the titre of ABO blood group antibodies at a low level and to inhibit B-cell activation (4-6). However, antibody-mediated graft rejection is still one of the major causes of poor outcome in the clinic.

Notably, in various ABOi-KT cases, the ABO blood group antibody returned to baseline levels without antibody-mediated graft rejection. For instance, Makroo *et al* found that after ABOi-KT the graft survived normally, even though the ABO blood group antibody titre returned to the preoperative level (7). Snell *et al* noted that the kidney graft survived normally for three years while the recipient did not receive any B-cell-targeted therapy, such as rituximab (8). In other words, immunological accommodation occurred in those cases.

Many researchers have found that the process of immunological accommodation is associated with changes in graft antigen and B-cell activation. After ABOi-KT, ABO blood group antigens on the endothelium of the kidney graft were found to be significantly decreased (9), and soluble ABO blood antigen produced by the transplanted kidney are able to bind to antibodies to reduce antibody-mediated immune reactions (10), thus the graft antigen may be one of the mechanisms underlying immunological accommodation. On the other hand, in ABOi-KT, B cells could be activated to produce enhancing antibodies (also named blocking antibodies) that bind competitively with ABO blood group antigens thus preventing an immune response (11). Consequently, ABO blood group antibodies play an important role in the process of immunological accommodation, but the mechanism of B-cell activation to produce antibodies with different functions is still unclear.

Previous research has identified that haptoglobin is associated with the activation of cytokine-induced killer cells (CIKs) (12), and B cells in peripheral blood mononuclear

cells (PBMCs) from blood group A could be activated by HK2 cells carrying blood group B antigen (13). In the present study, the role of HP in B-cell activation was analyzed by flow cytometry, dot-ELISA and bioinformatics. These results will provide novel insight for further research on immunological accommodation in ABOi-KT conditions.

Materials and methods

Peripheral blood was donated from blood group A volunteers after informed consent. The study was approved by the Animal Welfare and Research Ethics Committee of the Institute of University of South China (Hengyang, Hunan, China).

Cell culture. Peripheral blood mononuclear cells (PBMCs) from donors with blood group A were separated by the Ficoll density gradient method as reported by Huang *et al* (14). The HK2 cells (human, non-tumor cells), which carry human blood group B antigen, and PBMCs were divided into three groups: The HK2 group contained HK2 cells, the PBMC group contained PBMCs, and the HK2+PBMC group contained equal quantities of PBMCs and HK2 cells. All groups were cultured with RPMI-1640 medium (Thermo Fisher Scientific, Inc.) and 15% fetal calf serum (FCS; Thermo Fisher Scientific, Inc.) at 37˚C in 5% CO_2. In addition, previous research identified that B-cell activation is significantly upregulated to the highest level at day 4 (13). Thus, all of the cells were collected at day 4 for further detection.

Flow cytometric assay. Day 0 PBMCs were collected and divided into two groups: The isotype control group, which was mixed with 5 μl mouse IgG2α FITC-conjugated antibody (BD Biosciences; cat no. 2137834) and 5 μl mouse IgG1 APC-conjugated antibody (BD Biosciences; cat no. 39573); and the target group, which was mixed with 5 μl mouse anti-human CD3 FITC-conjugated antibody (BD Biosciences; cat. no. 4043995) and 5 μl mouse anti-human CD19 APC-conjugated antibody (BD Biosciences; cat. no. 555415). Both groups were incubated at room temperature for 15 min, and then treated with 200 μl Red Blood Cell Lysis Buffer (Solarbio) at room temperature for 10 min, and centrifuged at 150 x g for 10 min. Following resuspension with 1 ml physiological saline and centrifugation at 150 x g for 10 min, the cells were further treated following the instructions of the Cytofix/Cytoperm™ Fixation/Permeabilization Kit (BD Biosciences). In the process, 1 μl rabbit IgG monoclonal antibody (Abcam) was added to the isotype control group, and 2 μl rabbit anti-human HP (BosterBio) was added to the target group, and then the cells were respectively incubated with 1 μl PE-conjugated goat anti-rabbit IgG (4A Biotech Co., Ltd.). Finally, the cells were analyzed by BD FACS Aria II, and at least 100,000 cells were collected per sample.

Extraction of cell proteins and collection of supernatants. In the coculture system, HK2 cells were adhered on the bottom of a culture dish, while lymphocytes exhibited suspended growth. Thus, in the detection time, the lymphocytes were collected with the culture medium after being shaken lightly, and HK2 cells were collected using trypsinization (Solarbio). The supernatants of all groups were collected and centrifuged at 13,000 x g for 10 min and then transferred into clean EP tubes for further experiments. HK2 cells of the HK2 group, PBMCs of the PBMC group and the HK2+PBMC group were collected and then centrifuged at 900 x g for 10 min. The supernatants of all groups were collected and stored at -20˚C and the cells were resuspended in 2 ml physiological saline. Following centrifugation at 900 x g for 10 min, the cell pellet was collected and total protein was extracted using Native lysis Buffer (Solarbio). The total protein concentration was determined by a BCA Protein Assay Kit (Tiangen). HP concentration was detected by a Haptoglobin Kit (Dialab). All cell protein extracts were stored at -20˚C with 0.01 M phenylmethylsulfonyl fluoride (PMSF).

Table I. Primer sequences.

Primer	Sequence
HP-F	CAGCCAGAAACATAACCC
HP-R	TCTACACCCTAACTACTCCC
β-actin-F	ATCGTGCGTGACATTAAGGAG
β-actin-R	TAGGTGCTTTGATGGAAGTTGAG

F, forward; R, reverse.

Dot-ELISA analysis. A polyvinylidene fluoride (PVDF) membrane was activated with methyl alcohol for 15 sec and then soaked in TBS (Solarbio) for 10 min. For maintaining the comparability among different samples, the total protein concentration of culture supernatant was detected. After the PVDF membrane had dried at room temperature, 2 μl (0.5 μg/μl) protein of each sample was respectively applied to the PVDF membrane. Then, the membrane was blocked with blocking buffer (TBS containing 0.05% Tween-20 and 5% skim milk) at room temperature for 1 h. Following 3 washes with TBS for 5 min each, the membrane was incubated with rabbit anti-human HP (dilution 1:200; BosterBio; cat. no. BA3744) or mouse anti-human blood group B antibody (dilution 1:1,000; Abcam; cat. no. 3968) for 1 h at room temperature and rinsed 3 times with TBS for 5 min each time. The PVDF membrane was incubated with goat anti-rabbit IgG-HRP (dilution 1:5,000; Cell Signaling Technology; cat. no. 7074P2) or goat anti-mouse IgG-HRP (dilution 1:3,000; Thermo Fisher Scientific, Inc.; cat. no. 31431) for 40 min at room temperature. Finally, the membrane was rinsed 2 times with TBS for 15 min each. For the expression analysis of IgM and IgG, PVDF membranes were incubated with goat anti-human IgM-HRP (1:3,000; Thermo Fisher Scientific, Inc.; cat. no. 31415) or goat anti-human IgG-HRP (dilution 1:3,000; Thermo Fisher Scientific, Inc.; cat. no. 31410) for 1 h at room temperature and rinsed 2 times with TBS for 15 min each. All samples were dyed with ECL Plus (Solarbio) and analyzed by ChemiDoc XRS+ (Bio-Rad).

In addition, the interaction of HP and Smad3 was also analyzed using this method. In this experiment, rabbit anti-human Smad3 (dilution 1:200; BosterBio; cat. no. BA4559) and mouse anti-human Smad4 (dilution 1:200; BosterBio; cat. no. BM1601) were used as the primary antibodies, and goat anti-rabbit IgG-HRP (dilution 1:5,000;

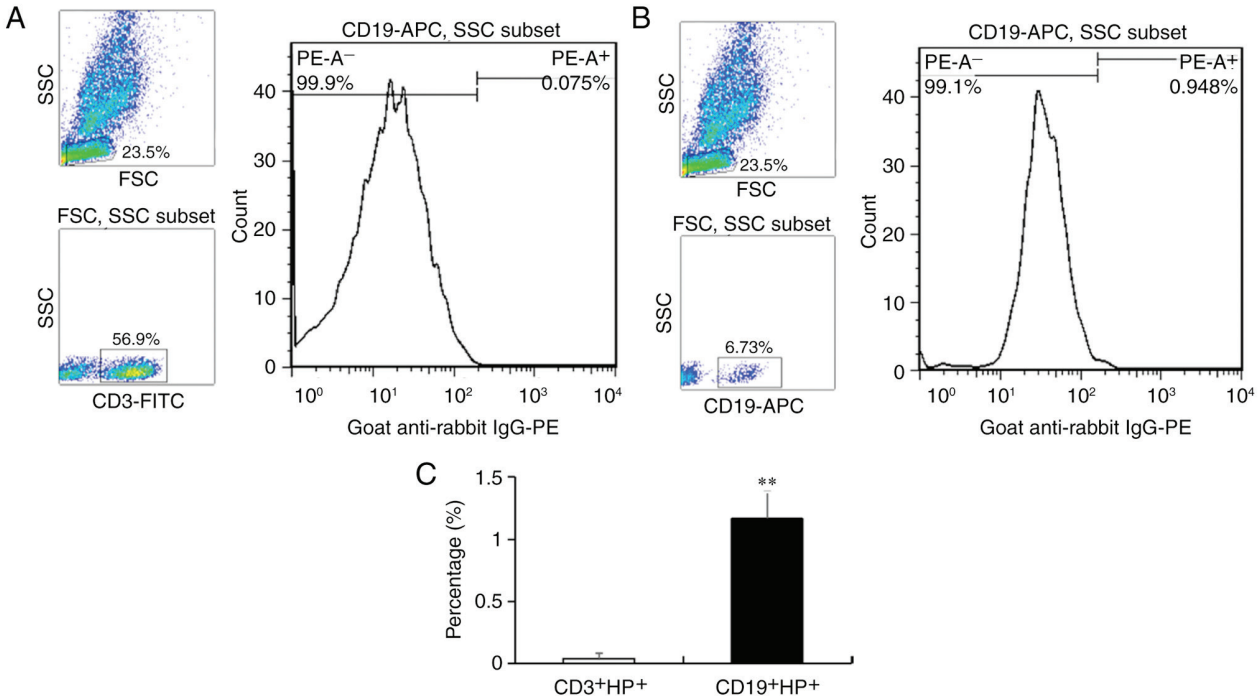

Figure 1. Flow cytometric analysis of HP expression in lymphocytes. (A) The expression of HP in $CD3^+$ cells. (B) The expression of HP in $CD19^+$ cells. Rabbit anti-human HP and PE-conjugated goat anti-rabbit IgG were used as the primary and secondary antibodies, respectively. (C) The average percentage of HP^+ cells in $CD3^+$ and $CD19^+$ lymphocytes. The starting samples of Fig. 1B was the same as those of Fig. 1A in this experiment. Bars represent the mean ± SD (n=6). $^{**}P<0.01$, significant difference. HP, haptoglobin.

Cell Signaling Technology; cat. no. 7074S) and goat anti-mouse IgG-HRP (dilution 1:5,000; Sigma-Aldrich; Merck KGaA; cat. no. AP308P) were used as the secondary antibodies. After 1 μl mouse anti-human HP (dilution 1:1,000; Abcam; cat. no. ab13429), rabbit anti-human HP (dilution 1:200), or mouse anti-human Smad4 (dilution 1:200; BosterBio) was applied to PVDF membranes, the membranes were blocked with blocking buffer at room temperature for 1 h. Following 3 washes with TBS for 5 min each, the cell protein extraction of the HK2+PBMC group (dilution 1:50) was added to the membranes and incubated at room temperature for 1 h and then rinsed 3 times with TBS for 5 min each. Then, the membranes were incubated with primary antibodies at room temperature for 1 h. After rinsing 3 times with TBS for 5 min each, the membranes were incubated with secondary antibodies at room temperature for 40 min. Finally, the membranes were rinsed 2 times with TBS for 15 min each, dyed with ECL Plus (Solarbio) and analyzed by ChemiDoc XRS+ (Bio-Rad).

RNA exaction and cDNA synthesis. After stimulation with HK2 cells for 4 days, cells from the PBMC and HK2+PBMC groups were collected, and the total RNA was extracted using an RNAsimple Total RNA kit (TianGen Biotech (Beijing) Co., Ltd.). cDNA was synthesized using a RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.).

PCR and sequence analysis. The primers used in this study are shown in Table I. The 40 μl premix for RT-qPCR was composed of 20 μl 2X PCR Master Mix (Thermo Fisher Scientific), 2 μl forward primer, 2 μl reverse primer, 2 μl cDNA template and 14 μl ddH_2O. The reaction programme was as follows: 94˚C for 2 min; 32 cycles at 94˚C for 30 sec, 55˚C for 30 sec, and 72˚C for 30 sec; and 72˚C for 10 min. A 15 μl PCR product was used to analyze the change in HP expression by agarose gel electrophoresis (1% agarose, 80 V for 1 h), and 25 μl PCR product of HP was used for sequencing.

Quantitative real-time PCR analysis. The primers are listed in Table I. The 20 μl premix for RT-qPCR was composed of 10 μl 2X SYBR Green PCR Mastermix (Beijing Solarbio Science & Technology Co., Ltd., China), 1 μl forward primer, 1 μl reverse primer, 1 μl cDNA template and 7 μl ddH_2O. The reaction programme was as follows: 95˚C for 2 min and 40 cycles at 95˚C for 5 sec and 60˚C for 30 sec. The experiment was detected by LightCycler 96 (Roche).

Bioinformatics analysis. Proteins interacting with HP were predicted by Cytoscape software 3.7.1 (https://cytoscape.org). Information on the HP interaction proteins was obtained from the National Center for Biotechnology Information (NCBI) website (https://www.ncbi.nlm.nih.gov/gene/?term=).

Statistical analysis. All experiments were repeated at least 3 times. The data were analyzed with SigmaPlot 12.0 software, and the results are shown as the mean ± SEM. Student's t-test was used to assess the statistical significance, and $P<0.05$ and 0.01 were considered to indicate significant and very significant differences, respectively.

Results

Analysis of lymphocytes expressing HP. To identify the type of lymphocytes that express HP, PBMCs were analyzed by flow cytometry (Fig. 1). To avoid the influence from the sample, the

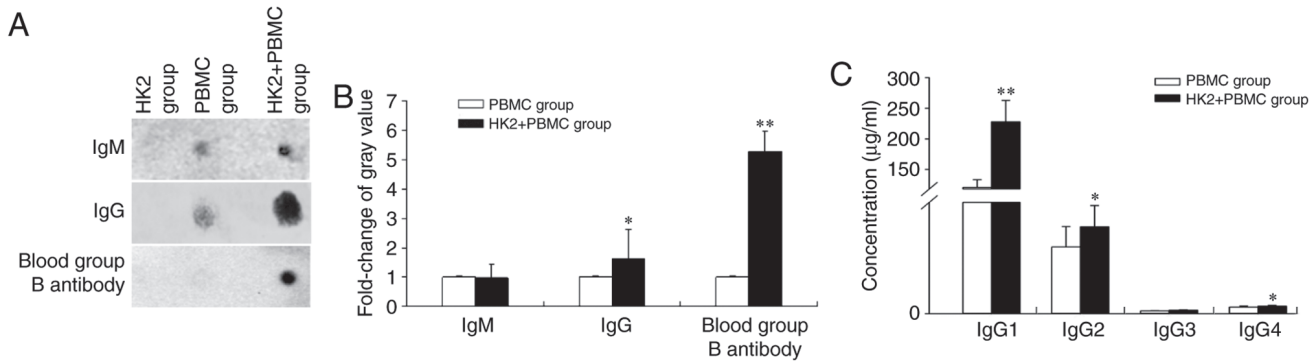

Figure 2. Expression of IgM, IgG, blood group B antibody and IgG subclasses by dot-ELISA and ELISA methods. (A) Dot-ELISA analysis of the expression of IgM, IgG, and blood group B antibody in cell culture supernatant of the PBMC and HK2+PBMC groups. (B) Grey values of the dot-ELISA results. (C) ELISA analysis of the expression of IgG subclasses in cell culture supernatants of the PBMC and HK2+PBMC groups. Bars represent the mean ± SD (n=3). *P<0.05 and **P<0.01, significant differences between the PBMC and HK2+PBMC groups. PBMC, peripheral blood mononuclear cell.

starting samples of Fig. 1B was the same as those of Fig. 1A in this experiment. The results showed that the percentage of $CD3^+$ T cells was 56.90% without an obvious HP^+ cell population (Fig. 1A), and the percentage of $CD19^+$ B cells was 6.73% and that of HP^+ cells was 0.95% (Fig. 1B). After statistical analysis, the average percentage of $CD19^+HP^+$ cells was calculated to be 1.16%. These results indicated that $CD19^+$ B cells were the lymphocytes that expressed HP.

Expression of blood group B antibody, IgM, total IgG and IgG subclasses. Human blood group antibodies are divide into two major groups: IgM and IgG (15). Thus, in this study, the expression of IgM, IgG, blood group B antibody and IgG subclasses in cell culture supernatants were detected after co-culture with HK2 cells. Dot-ELISA analysis showed that IgG and blood group B antibody levels were significantly increased 1.63- and 5.28-fold, respectively, and IgM was not significantly altered (Fig. 2A and B). Moreover, an ELISA assay showed that IgG1, IgG2 and IgG4 were increased 1.89-, 1.30- and 1.16-fold, respectively, and IgG3 was increased 1.21-fold, which was not statistically significant (Fig. 2C, Table II). These results indicated that the B cells were activated to produce antibodies with different biological activities.

Expression of HP at the mRNA and protein levels. As shown in Fig. 3, the expressed sequence tags (EST) of HP were highly similar to the sequence of human HP (Fig. 3A and B). After B cells were activated by HK2 cells, HP mRNA was significantly downregulated 14.38-fold (Fig. 3C and D). Moreover, dot-ELISA assay revealed that HP was detected in the cell protein extract of the PBMC and HK2+PBMC groups but not in the cell protein extract of the HK2 cells or in the culture supernatant of any of the groups (Fig. 3E). In addition, the concentration of HP in the HK2+PBMC group (0.069 mg/ml) was significantly increased 1.34-fold compared with that in the PBMC group (0.052 mg/ml; Fig. 3F). These results indicated that HP was associated with the activation of B cells.

Analysis of the interaction protein of HP. To reveal the signaling pathway of HP in B cells, bioinformatics analysis and dot-ELISA were performed. The results showed that of the

Table II. ELISA was used to detect the concentration of IgG subclasses in PBMC and HK2+PBMC groups.

IgG subclasses	PBMC group	HK2+PBMC group
IgG1 (μg/ml)	120.45±12.71	227.85±34.94
IgG2 (μg/ml)	2.39±0.73	3.12±0.77
IgG3 (μg/ml)	0.1±0.01	0.12±0.02
IgG4 (μg/ml)	0.23±0.05	0.27±0.03

PBMC, peripheral blood mononuclear cell.

24 potential proteins that interacted with HP, Smad3 (4088), which possessed higher indegree and edge betweenness, was selected as the main candidate (Fig. 4A). The protein-protein interaction network indicated that Smad3 could interact with other SMAD family proteins such as Smad4 (4089, Fig. 4B). Furthermore, dot-ELISA verified that the HP-Smad3 and Smad4-Smad3 complexes were detected in the cell protein extraction of PBMCs (Fig. 4C and E) but not the HP-Smad4 complex (Fig. 4D). These results indicated that Smad3 was the target protein that interacted with HP.

Discussion

The present study found that HP was mainly expressed by B cells and not by T cells (Fig. 1). The results were similar to studies that demonstrated that HP is an important activator of immune cells (16-18). Based on these results, we conjectured that HP plays important roles in B-cell activation.

To analyze the activation of B cells, IgM, IgG and blood group B antibody were detected using Dot-ELISA. The results showed that the antibodies were not detected in the sample from HK2 cells. Meanwhile, B-cell activation was detected by dot-ELISA and ELISA methods. Dot-ELISA showed that IgG and blood group B antibodies were all significantly increased while IgM was not significantly altered (Fig. 2A and B), and ELISA results showed that IgG1, IgG2 and IgG4 were all significantly upregulated (Fig. 2C). Ig is the main effector molecule of humoral immunity, and its concentration is related to the number of B cells (19). That is, B cells in the

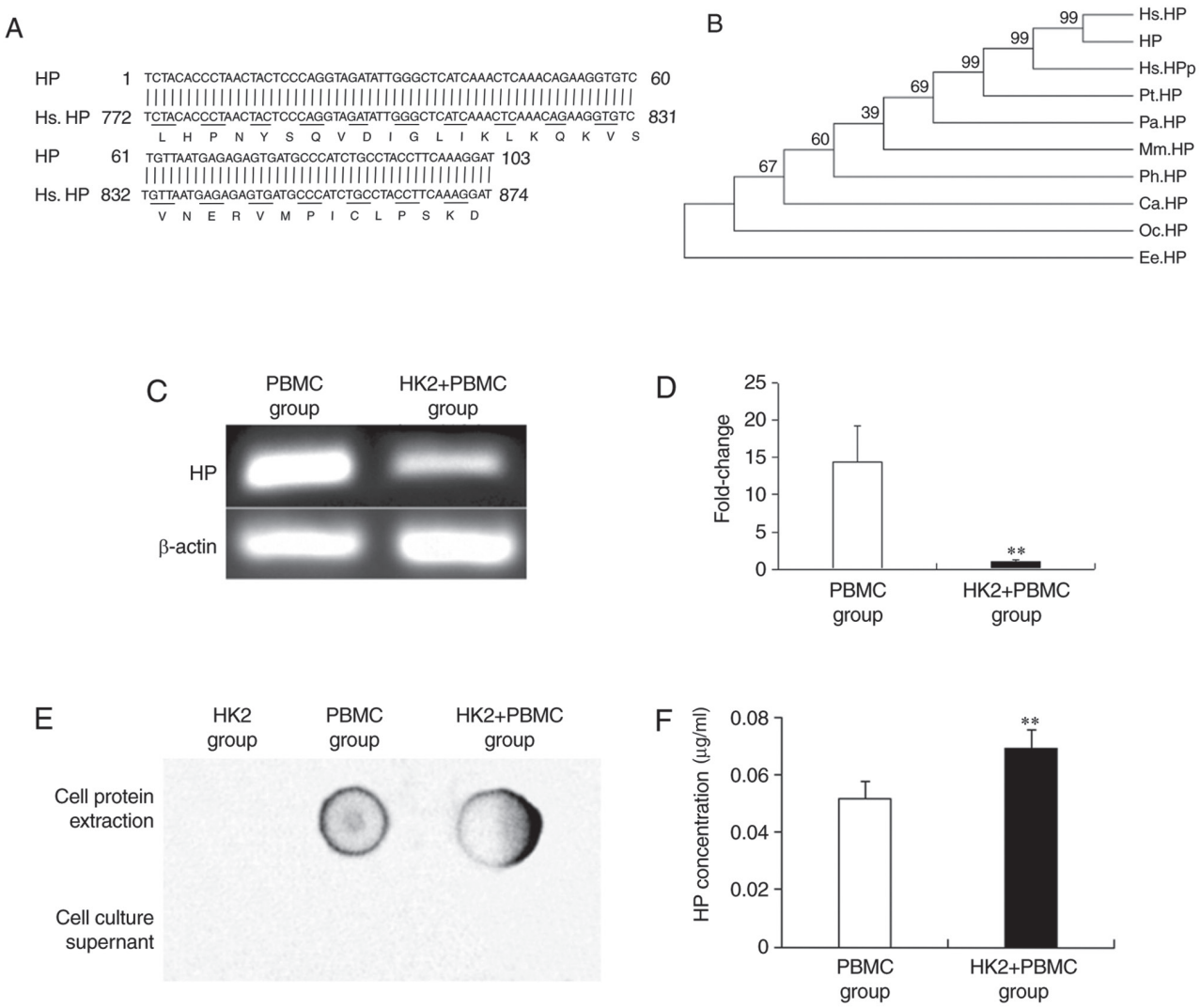

Figure 3. Change in HP expression in B cells of the PBMC and HK2+PBMC groups. (A) Sequence analysis of the HP EST. (B) Phylogenetic tree analysis of HP. Hs, *Homo sapiens*; Pt, *Pan troglodytes*; Ph, *Papio hamadryas*; Ca, *Cercocebus atys*; Pa, *Pongo abelii*; Mm, *Microcebus murinus*; Oc, O*ryctolagus cuniculus*; Ee, *Elephantulus edwardii*; HP, haptoglobin; HPP, haptoglobin isoform 3 preproprotein. (C) Agarose gel electrophoresis analysis of the expression of HP mRNA. (D) RT-qPCR analysis of the expression of HP mRNA. (E) Dot-ELISA analysis of the expression of HP. Rabbit anti-human HP (dilution 1:200) and goat anti-rabbit IgG-HRP (dilution 1:5,000) antibodies were used as the primary and secondary antibodies, respectively. (F) Immunoturbidimetric analysis of the concentration of HP. Bars represent the mean ± SD (n=3). **P<0.01, significant differences between the PBMC and HK2+PBMC groups. PBMC, peripheral blood mononuclear cell; HP, haptoglobin.

ABOi kidney cell model were activated to produce different functional antibodies.

Following these results, the expression of HP was analyzed at the mRNA and protein levels. After the EST sequence of HP was verified to be that of human HP (Fig. 3A and B), PCR and RT-qPCR analyses showed that HP mRNA was significantly downregulated (Fig. 3C and D). In contrast to the change in HP mRNA levels, HP protein was only detected in PBMCs by dot-ELISA (Fig. 3E), and the concentration was significantly upregulated after HK2 stimulation (Fig. 3F). As Liu *et al* reported (20), the changes in gene expression at the mRNA and protein levels are not always in line. Therefore, the results suggested that HP was closely related to B-cell activation.

In addition, further research was carried out to explore the possible mechanism by which HP is involved in B-cell activation. Bioinformatics analysis found that HP had 24 interaction proteins (Fig. 4A). Among them, Smad3 (4088) was selected as the most likely interaction protein because of its higher indegree and edge betweenness. Smad3 is associated with the regulation, differentiation and survival of cells because it is a key signal transducer in multiple signalling pathways (21-23). One example is the classic TGF-β signaling pathway, in which Smad3 is a major downstream mediator of TGF-β and is phosphorylated by the activated TGF-β receptor (24,25). After phosphorylation, Smad3 is directly involved in Ig class switch recombination in B cells, which allows the production of antibodies of different subtypes and subclasses (26,27). Most important for this study, the HP-Smad3 complex was detected in the cell protein extract by the Dot-ELISA method (Fig. 4C-E). To ensure the reliability of the results of Dot-ELISA, multiple negative and positive controls were selected in this study. For example, Smad3 and Smad4 were identified with interaction (28), thus they were selected as positive controls; Bio-informatic analysis showed that there is no interaction between HP and Smad4, thus they were selected

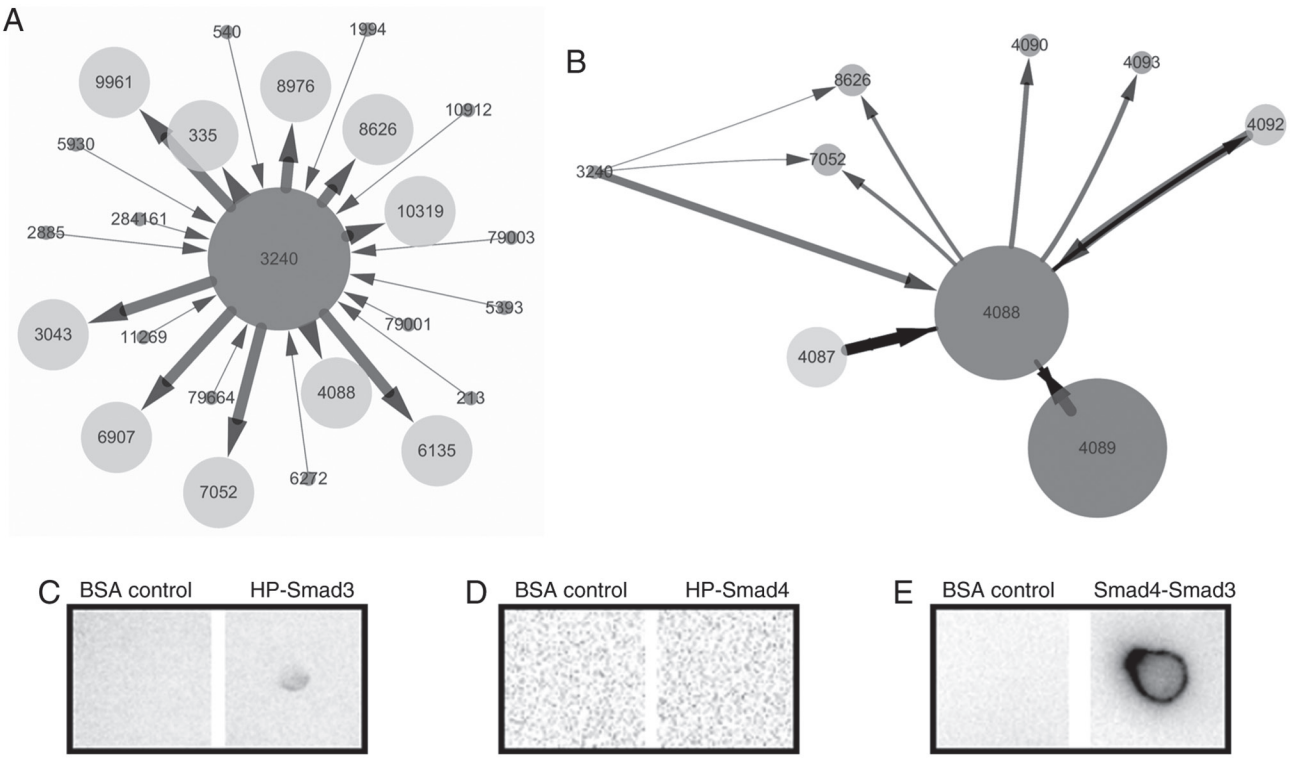

Figure 4. Analysis of the interaction protein of HP. (A) Bioinformatics analysis of protein interactions with HP (3240). The numbers in the figure are the Gene IDs obtained from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/gene/?term=). The indegree is indicated by the size of the nodes. The edge betweenness and shared interaction are indicated with the width and length of the lines, respectively. The direction of interaction is indicated with an arrow. (B) The protein-protein interaction network of HP and Smad3 (4088). (C) Interaction analysis of HP and Smad3. PVDF membranes were blotted with mouse anti-human HP (dilution 1:1,000), followed by incubation with cell protein extract of the HK2+PBMC group, rabbit anti-human Smad3 (dilution 1:200) and goat anti-rabbit IgG-HRP (dilution 1:5,000). (D) Interaction analysis of HP and Smad4. PVDF membranes were blotted with rabbit anti-human HP (dilution 1:200), followed by incubation with cell protein extract of the HK2+PBMC group, mouse anti-human Smad4 (dilution 1:200) and goat anti-mouse IgG-HRP (dilution 1:5,000). (E) Interaction analysis of Smad3 and Smad4. PVDF membranes were blotted with rabbit anti-human Smad3 (dilution 1:200), followed by incubation with cell protein extract of the HK2+PBMC group, mouse anti-human Smad4 (dilution 1:200) and goat anti-mouse IgG-HRP (dilution 1:5,000). The experiments were repeated at least 3 times. PBMC, peripheral blood mononuclear cell; HP, haptoglobin.

as a negative control. In addition, the BSA group was the negative control for the three groups. After comparison of those control groups, we confirmed that the result of HP combined with Smad3 was reliable.

In conclusion, the results of this study indicate that HP is involved in B-cell activation via interaction with Smad3. This research provides novel insight on immunological accommodation in ABOi-KT, and further research will explore whether the HP-Smad3 complex affects B-cell activation via the TGF-β signaling pathway.

Acknowledgements

Not applicable.

Funding

This study was supported by the Zhengxiang Scholar Program of The University of South China (Hengyang, Hunan, China).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Laboratory experiments, data analysis and manuscript writing were accomplished by JC and CC. Manuscript revision and data analysis were accomplished by LL, YZ and HZ. The guidance of the experimental design was performed by JX and YW. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The study was approved by the Animal Welfare and Research Ethics Committee of the Institute of University of South China. Informed consent was received from the volunteers.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Koo TY and Yang J: Current progress in ABO-incompatible kidney transplantation. Kidney Res Clin Pract 34: 170-179, 2015.
2. Takahashi K and Saito K: ABO-incompatible kidney transplantation. Transplant Rev (Orlando) 27: 1-8, 2013.
3. Takahashi K, Tanabe K, Ooba S, Yagisawa T, Nakazawa H, Teraoka S, Hayasaka Y, Kawaguchi H, Ito K, Toma H, *et al*: Prophylactic use of a new immunosuppressive agent, deoxyspergualin, in patients with kidney transplantation from ABO-incompatible or preformed antibody-positive donors. Transplant Proc 23: 1078-1082, 1991.
4. Muramatsu M, Gonzalez HD, Cacciola R, Aikawa A, Yaqoob MM and Puliatti C: ABO incompatible renal transplants: Good or bad? World J Transplant 4: 18-29, 2014.
5. Morimoto H, Ide K, Tanaka Y, Ishiyama K, Ohira M, Tahara H, Akita T, Tanaka J and Ohdan H: Different sensitivity of rituximab-treatment to B-cells between ABO-incompatible kidney and liver transplantation. Hum Immunol 77: 456-463, 2016.
6. Lee KW, Park JB, Oh DK, Na BG, Choi JY, Cho WT, Lee SH, Park HJ, Cho D, Huh WS and Kim SJ: Short-term outcomes of ABO-incompatible living donor kidney transplantation with uniform protocol: Significance of baseline anti-ABO titer. Transplant Proc 48: 820-826, 2016.
7. Makroo RN, Nayak S, Chowdhry M, Jasuja S, Sagar G, Rosamma NL and Thakur UK: Role of therapeutic plasma exchange in reducing ABO titers in patients undergoing ABO-incompatible renal transplant. Apollo Medicine 13: 31-36, 2016.
8. Snell GI, Davis AK, Menahem S, Kotecha S, Whitford HM, Levvey BJ, Paraskeva M, Webb A, Westall GW and Walker RG: ABO incompatible renal transplantation following lung transplantation. Transpl Immunol 39: 30-33, 2016.
9. Tanabe T, Ishida H, Horita S, Yamaguchi Y, Toma H and Tanabe K: Decrease of blood type antigenicity over the long-term after ABO-incompatible kidney transplantation. Transpl Immunol 25: 1-6, 2011.
10. Kim J, Kim S, Hwang IS, Choi JR, Lee JG, Kim YS, Kim MS and Kim HO: Effects of neutralization by soluble ABH antigens produced by transplanted kidneys from ABO-incompatible secretor donors. Ann Lab Med 37: 254-260, 2017.
11. Chen C and Guo H: Transplantation pathology. People's Medical Publishing House (edition 1), Beijing, China, 2009.
12. Cao J, Chen C, Gao Y, Hu L, Liang Y and Xiao J: Identification of a protein associated with the activity of cytokine-induced killer cells. Oncol Lett 14: 6937-6942, 2017.
13. Cao J, Liu L, Zhang Y, Xiao J and Wang Y: The influence of HK2 blood group antigen on human B cell activation for ABOi-KT conditions. BMC Immunol 18: 49, 2017.
14. Huang J, Kan Q, Lan, Zhao X, Zhang Z, Yang S, Li H, Wang L, Xu L, Cheng Z and Zhang Y: Chemotherapy in combination with cytokine-induced killer cell transfusion: An effective therapeutic option for patients with extensive stage small cell lung cancer. Int Immunopharmacol 46: 170-177, 2017.
15. Khalili I, Koch M, Thaiss F, Plaetke R, Bruegger J and Peine S: Systematic comparison of IgM and IgG ABO antibody titers by using tube and gel card techniques and its relevance for ABO-incompatible kidney transplantation. Clin Lab 63: 1393-1401, 2017.
16. Tan W, Wang F, Guo D, Ke Y, Shen Y, Lv C and Zhang M: High serum level of haptoglobin is associated with the response of 12 weeks methotrexate therapy in recent-onset rheumatoid arthritis patients. Int J Rheum Dis 19: 482-489, 2016.
17. Delanghe JR, Langlois MR and De Buyzere ML: Haptoglobin polymorphism: A key factor in the proatherogenic role of B cells? Atherosclerosis 217: 80-82, 2011.
18. Huntoon KM, Russell L, Tracy E, Barbour KW, Li Q, Shrikant PA, Berger FG, Garrett-Sinha LA and Baumann H: A unique form of haptoglobin produced by murine hematopoietic cells supports B-cell survival, differentiation and immune response. Mol Immunol 55: 345-354, 2013.
19. Huang J, Doria-Rose NA, Longo NS, Laub L, Lin CL, Turk E, Kang BH, Migueles SA, Bailer RT, Mascola JR and Connors M: Isolation of human monoclonal antibodies from peripheral blood B cells. Nat Protoc 8: 1907-1915, 2013.
20. Liu Y, Beyer A and Aebersold R: On the dependency of cellular protein levels on mRNA abundance. Cell 165: 535-550, 2016.
21. Hao Y, Yang X, Zhang D, Luo J and Chen R: Long noncoding RNA LINC01186, regulated by TGF-β/SMAD3, inhibits migration and invasion through Epithelial-Mesenchymal-Transition in lung cancer. Gene 608: 1-12, 2017.
22. Letterio JJ: Murine models define the role of TGF-beta as a master regulator of immune cell function. Cytokine Growth Factor Rev 11: 81-87, 2000.
23. DiRenzo DM, Chaudhary MA, Shi X, Franco SR, Zent J, Wang K, Guo LW and Kent KC: A crosstalk between TGF-β/Smad3 and Wnt/β-catenin pathways promotes vascular smooth muscle cell proliferation. Cell Signal 28: 498-505, 2016.
24. Kashiwagi I, Morita R, Schichita T, Komai K, Saeki K, Matsumoto M, Takeda K, Nomura M, Hayashi A, Kanai T and Yoshimura A: Smad2 and Smad3 inversely regulate TGF-β autoinduction in Clostridium butyricum-activated dendritic cells. Immunity 43: 65-79, 2015.
25. Lan HY: Smads as therapeutic targets for chronic kidney disease. Kidney Res Clin Pract 31: 4-11, 2012.
26. McKarns SC, Letterio JJ and Kaminski NE: Concentration-dependent bifunctional effect of TGF-beta 1 on immunoglobulin production: A role for Smad3 in IgA production in vitro. Int Immunopharmacol 3: 1761-1774, 2003.
27. Zhang Y and Derynck R: Transcriptional regulation of the transforming growth factor-beta-inducible mouse germ line Ig alpha constant region gene by functional cooperation of Smad, CREB, and AML family members J Biol Chem 275: 16979-16985, 2000.
28. Chacko BM, Qin BY, Tiwari A, Shi G, Lam S, Hayward LJ, De Caestecker M and Lin K: Structural basis of heteromeric smad protein assembly in TGF-beta signaling. Mol Cell 15: 813-823, 2004.