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Role of HLA-G1 in trophoblast cell proliferation, adhesion and invasion

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ABSTRACT

Trophoblast cells are important in embryo implantation and fetomaternal tolerance. HLA-G is specifically expressed at the maternal–fetal interface and is a regulator in pregnancy. The aim of the present study was to detect the effect of HLA-G1 on trophoblast cell proliferation, adhesion, and invasion. Human trophoblast cell lines (JAR and HTR-8/SVneo cells) were infected with HLA-G1-expressing lentivirus. After infection, HLA-G1 expression of the cells was detected by western blotting. Cell proliferation was detected by the BrdU assay. The cell cycle and apoptosis of JAR and HTR-8/SVneo cells was measured by flow cytometry (FCM). The invasion of the cells under different conditions was detected by the transwell invasion chamber assay. HLA-G1 didn't show any significant influence on the proliferation, apoptosis, adhesion, and invasion of trophocytes in normal culture conditions. However, HLA-G1 inhibited JAR and HTR-8/SVneo cells invasion induced by hepatocyte growth factor (HGF) under normal oxygen conditions. In conditions of hypoxia, HLA-G1 couldn't inhibit the induction of cell invasion by HGF. HLA-G1 is not an independent factor for regulating the trophocytes. It may play an indirect role in embryo implantation and formation of the placenta.

1. Introduction

Pregnancy is a highly regulated physiological process which starts from successful embryo implantation and the formation of fetomaternal tolerance. Invasive trophoblast cells and the extracellular matrix (ECM) are crucial in the process of normal implantation [1]. The appearance of trophocytes in the blastocyst stage indicates that the embryo has the capacity to invade into the endometrium. Then, trophoblasts regulate the differentiation, proliferation, and adhesion of endometrial stromal cells during implantation; they are also important for sustaining placental development [2]. Fetomaternal tolerance is another key factor of successful gestation, as it can protect the embryo from attack by the maternal immune system. Previous studies have proven that trophoblasts play an important role in maintaining fetomaternal tolerance [3,4]. Both the embryo implantation and the fetomaternal tolerance are regulated by many molecules, such as CD4, CD5 and major histocompatibility complex (MHC) [5–7].

HLA-G is a kind of MHC class Ib molecule including eight protein isoforms (named HLA-G1-8) [8]. HLA-G is specifically expressed at the maternal–fetal interface and is associated with fetomaternal tolerance [5]. It has been reported that HLA-G is an immunosuppressor that is used to modulate the local immune response and suppress the attack of the maternal immune system against the fetus [9]. This suppression is achieved by affecting maternal cytokine secretion to control the invasion of trophoblastic cells [10]. Unusual expression of HLA-G or HLA-G dysfunction can change the microenvironment at the maternal–fetal interface, leading to the occurrence of various pathological pregnancies. For instance, low expression of HLA-G had a close relationship with recurrent spontaneous abortion and preeclampsia [11]. HLA-G level was lower among pregnant woman with preeclampsia compared with normal pregnant women [12–16]. It has been proven that HLA-G1 inhibited the proliferation of CD4+ T cells and the activity of autoreactive T cells to maintain the maternal–fetal immune tolerance [17–22]. Natural killer (NK) cells are the main cells in the

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microenvironment at the maternal–fetal interface, accounting for 70% of immune cells [23]. HLA-G1 could bind to the inhibitory receptor p49 which is expressed on the surface of NK cells to protect the embryo [24]. All of the above information indicates that HLA-G1 plays an important role in embryo implantation and maternal–fetal immune tolerance. However, the mechanism is still not clear.

In the present study, we detected the influence of HLA-G1 on trophoblast proliferation, adhesion, and invasion. The results showed that HLA-G1 did not affect the proliferation and adhesion of trophoblasts, while HLA-G1 could inhibit trophoblast invasion induced by hepatocyte growth factor (HGF), and the effect was decreased under hypoxia. The above results indicate that HLA-G1 might be involved in regulating trophoblast activity together with other factors in the process of embryo implantation.

2. Materials and methods

2.1. Cell culture

JAR, JEG-3, and HTR-8/SVneo cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA). ECC-1 cell line and human primary endometrial cell were kindly provided by Dr. Y. Zhang (The State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China). Briefly, all cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mmol/l L-glutamate, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were cultured at 37 °C in a 5% CO_2 incubator.

2.2. Lentiviral vector production

Lentiviral HLA-G1-expressing vectors were provided by Dr. H.X. Zhao (Tangdu Hospital, Xian, China). Nine micrograms of lentiviral expression vectors, 12 μg of packaging vectors pCMVR (Tiangen Biotech co., Ltd., Beijing, China), and 3 μg of pMDG (Tiangen Biotech co., Ltd., Beijing, China) were mixed and incubated with 293 T cells at 37 °C, 5% CO_2 for 48 h. The cell supernatants were collected, and then concentrated using a 0.45 μm filter (Amicon Ultra-15 100 K, Millipore, Billerica, MA). The viral titer was calculated using the serial dilution method, and recombinant virus was stored at −70 °C until used.

2.3. Infection with HLA-G1-expressing lentivirus

JAR and HTR-8/SVneo cells in the logarithmic growth phase were cultured with the concentrated lentivirus solution (30 μl per well). Infections were performed for 6 h in RPMI-1640 medium containing 5 μg/ml polybrene (Sigma–Aldrich), then supplemented with lentiviral vectors (30 μl per well) and cultured for another 6 h at 37 °C. The medium was removed and RPMI-1640 medium without virus was added. These infected JAR and HTR-8/SVneo cells were cultured for 2–4 days.

2.4. Western blotting analysis

JAR, HTR-8/SVneo, and JEG-3 cells were homogenized and lysed with RIPA lysis buffer. Protein concentration was assayed using the micro-BCA protein assay kit (Pierce, Rockford, IL). Proteins (20–30 μg per lane) were separated by 10% SDS-PAGE and transfected onto a nitrocellulose membrane (Amersham Pharmacia, Germany). Then, nonspecific binding was blocked by incubating with 5% nonfat milk in PBST buffer at room temperature for 1 h. The membrane was incubated with 1:1000 dilution of primary antibodies against HLA-G1 and β-actin (Sigma, CA) at 4 °C overnight. The membrane was washed five times with PBST buffer. Goat anti-mouse immunoglobulin (IgG; 1:5000, Sigma, CA) was added and incubated at room temperature for 1 h. Chemiluminescent detection was performed using a Bio-Rad ChemiDoc MP Imaging System.

2.5. Cell proliferation assay

A BrdU cell proliferation assay kit (Millipore, Billerica, MA, USA) was used to detect the cell proliferation. JAR and HTR-8/SVneo cells were cultured in 96-well plates for 48 h, and 20 μl of BrdU solution was added per well and incubated for 2 h. The medium was removed and 200 μl/well of the fixing solution was added and incubated at room temperature for 30 min. Then, the solution was removed and 100 μl/well prepared detection antibody solution was added and incubated for 1 h at room temperature. After that, the plates were washed three times with wash buffer followed by the addition of 100 μl/well of prepared Horse Reddish Peroxidase (HRP)-conjugated secondary antibody solution and incubated for 30 min at room temperature. Then, the plates were washed three times with wash buffer and 100 μl of tetramethylbenzidine (TMB) substrate was added and incubated for 30 min at room temperature. The amount of BrdU incorporated into the cells was determined at 450 nm by a micro-plate reader (Bio-Rad, Hercules, CA).

2.6. Cell cycle analysis

Cell cycle distribution was analyzed using flow cytometry (FCM). Briefly, JAR and HTR-8/SVneo cells were trypsinized at 24 h after infection, washed with PBS and fixed with 70% ethanol. Fixed cells were washed with PBS and incubated with 20 μg/ml RNase for 30 min before they were stained by Propidium Iodide (PI) (Sigma, CA). The cells were then analyzed by FCM (Becton–Dickinson, NJ).

2.7. Cell apoptosis analysis

Cell apoptosis was analyzed by FCM using an Annexin V-FITC-PI Apoptosis Detection Kit (Abcam, Cambridge, UK). Briefly, JAR and HTR-8/SVneo cells were collected at 24 and 48 h after infection, washed with PBS, and suspended with 500 μl binding buffer. The cells were incubated with Annexin V-FITC at room temperature for 10 min and stained by PI, and then analyzed by FCM for relative quantitative apoptosis.

2.8. Cell adhesion assay

The CytoSelect™ 48-well cell adhesion assay kit (Cell Biolabs, Inc., San Diego, CA) was used to detect the adhesion of JAR and HTR-8/SVneo cells according to the manufacturer's instructions. Briefly, 48-well tissue culture plates were primarily packed with six components of ECM (fibronectin [Fn], fibrillin, laminin, type I collagen, type IV collagen, and bovine serum albumin) separately. The plates were incubated for 10 min at 37 °C, then cells (1×10^5) were seeded in the wells, and incubated for 75 min at 37 °C. The medium was removed and 200 μl per well of cell staining solution was added and incubated for 10 min. Then, the staining solution was removed and 200 μl of TMB substrate was added and incubated for 10 min. In total, 150 μl of solution was sucked up and transferred into 96-well tissue culture plates. The absorbance at 578 nm was measured with a micro-plate reader.

For another adhesion assay, a cell counting kit-8 (CCK-8; Dojindo, Gaithersburg, MD) was used. ECC-1 and primary endometrial cells were used as the basement membrane to detect the effect of HLA-G1 on the adhesion of JAR and HTR-8/SVneo cells. Briefly, ECC-1 and primary endometrial cells were packed in 96-well tissue culture plates, and infected cells were seeded in the

wells and incubated for 2 h at 37 °C. The plates were washed three times and 10 μl of CCK-8 solution was added to each well. The plates were further incubated for 4 h at 37 °C before the absorbance measurement at 450 nm.

2.9. Cell invasion assay

The transwell invasion chamber assay was used to detect cell invasion. The bottom of the transwell invasion chamber (Corning, NY) is made of a polycarbonate membrane with 8 μm membrane pores. An appropriate amount of matrigel (BD Biosciences, Bedford MA) was used to cover the surface of the polycarbonate membrane to produce an artificial base membrane and divide the chamber into the upper and lower parts. Each group of cells (2×10^5 cells suspended in 180 μl of serum-free RPMI-1640 medium) was seeded into the upper chamber of the transwell invasion system, and 600 μl of RPMI-1640 medium containing 10% fetal bovine serum was added to the lower chamber. The chamber was then placed in an incubator for 24 h. The upper chamber was then removed, and the medium was discarded. Cells on the upper surface of the base membrane were removed with a sterile cotton swab. Cells transferred to the lower surface of the base membrane were stained with hematoxylin and eosin (Sigma, CA), and the number of cells was counted under a Leica microscope (Precise, Beijing, China). Five fields were randomly selected for the calculation of each sample, and the experiment was repeated three times. For the HGF-positive group, 600 μl serum-supplemented medium containing 20 ng/ml of HGF was added to the 24-well plates. The cells of the hypoxic group were cultured at 37 °C, 5% CO_2, 3% O_2 for 24 h.

2.10. Statistical analysis

Statistical analysis was performed using SPSS 13.0 software (SPSS, Chicago, IL). All data are presented as mean ± SD. The

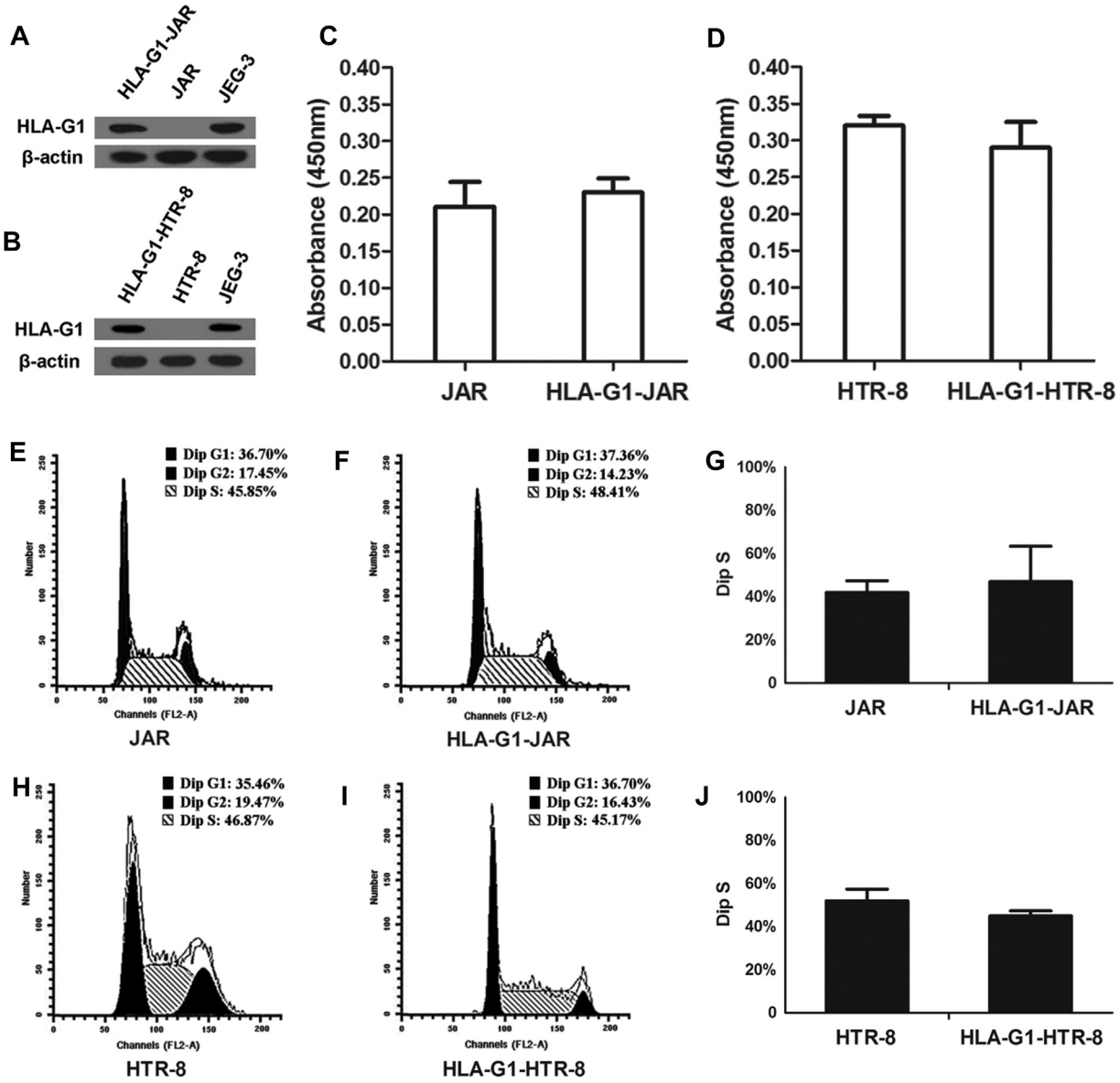

Fig. 1. Effect of HLA-G1 on cell proliferation and cell cycle. JAR and HTR-8/SVneo cells were infected with HLA-G1-expressing lentivirus. Expression of HLA-G1 was detected by western blotting. The cell proliferation was detected by the BrdU assay. Cell cycle was measured by FCM. (A–B) Expression of HLA-G1 in different cells. (C–D) Cell proliferation JAR and HTR-8/SVneo cells. (E–G) Cell cycle of JAR cells. (H–J) Cell cycle of HTR-8/SVneo cells. Data are represented as mean ± SD of three dependent experiments.

statistical significance of differences among groups was evaluated with Dunnett's test subsequent to analysis of variance (ANOVA). The statistical analysis between two groups was performed using an t test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of HLA-G1 on cell proliferation and cell cycle

After infection with HLA-G1-expression lentivirus, the HLA-G1 expression of JAR and HTR-8/SVneo cells was detected by western blotting. As shown in Fig. 1A and B, HLA-G1 was expressed in the infected cells. Cell proliferation of JAR and HTR-8/SVneo cells was determined by BrdU assay. The results of the assays showed that the proliferation of JAR cells was not influenced by HLA-G1 (Fig. 1C). In addition, the proliferation of HTR-8/SVneo cells which were infected with HLA-G1-expression lentivirus also exhibited no difference with the control group (Fig. 1D).

Cell cycle of the JAR and HTR-8/SVneo cells was detected by FCM. As shown in Fig. 1E–G, the percentage of S phase cells was (48.31 ± 4.70)% in HLA-G1-JAR and (45.85 ± 4.56)% in JAR. The results showed no significant difference between HLA-G1-JAR and control JAR cells ($P > 0.05$). The percentage of S phase cells in HLA-G1-HTR-8 cells was also similar to that in HTR-8 cells ($P > 0.05$) (Fig. 1H–J).

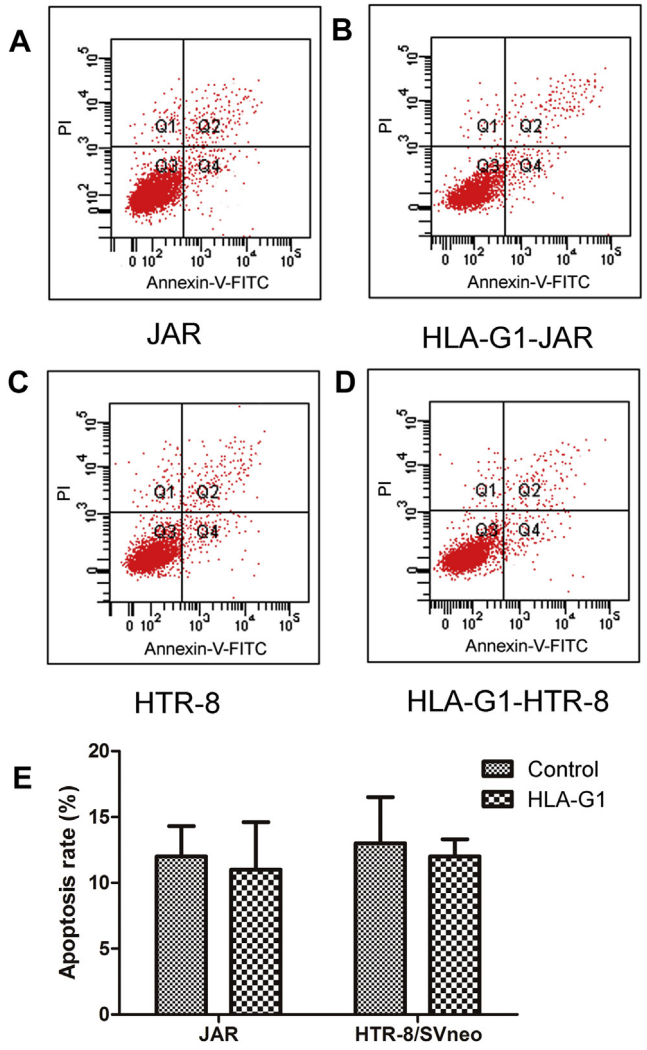

Fig. 2. Effect of HLA-G1 on cell apoptosis. (A–B) Cell apoptosis of JAR and HTR-8/SVneo cells was measured by FCM. (E) Quantitative description of cell apoptosis. Data are represented as mean ± SD of three dependent experiments.

3.2. Effect of HLA-G1 on cell apoptosis

In certain situations, the decrease in cell proliferation is caused by an increase of cell apoptosis; on the contrary, it may be influenced by decrease of cell apoptosis. In this study, the influence of HLA-G1 on JAR and HTR-8/SVneo cells apoptosis was detected by FCM. The results showed that the cell apoptosis was not significantly different between HLA-G1-JAR and JAR cells (Fig. 2) ($P > 0.05$). In the HTR-8/SVneo cells, the difference in cell apoptosis between HTR-8 and HLA-G1-HTR-8 was also not significant. Therefore, we can eliminate the impact of apoptosis on proliferation.

3.3. Effect of HLA-G1 on cell adhesion

Six components of the ECM were selected to detect the adhesion of JAR and HTR-8/SVneo cells. The results showed that adhesion of the two cell lines had a close relationship with Fn, while it showed no significant interaction with other components of the ECM (Fig. 3A and B). However, it seemed that HLA-G1 has no significant effect on the adhesion of the cells ($P > 0.05$).

Embryo implantation started from the contact between trophoblast cells and endometrial cells [25]. The adhesive attraction between trophoblast cells (JAR and HTR-8/SVneo) and endometrial cells (ECC-1 and primary endometrial cells) was detected in this study. However, no difference was found in adhesion between HLA-G1-expressing and normal cells ($P > 0.05$) (Fig. 3C and D). This indicated that HLA-G1 did not play a direct role in the adhesive attraction of trophoblast cells and endometrial cells.

3.4. Effect of HLA-G1 on cell invasion

The invasion of trophocytes plays a key role in the process of embryo implantation. In this study, the invasion of JAR and HTR-8/SVneo cells (with/without HGF induction) in normal oxygen/hypoxia conditions was detected by the transwell invasion chamber assay. In normal oxygen conditions, the invasion of JAR cells which expressed HLA-G1 showed no significant difference with the control JAR cells (Fig. 4I). The invasion of the control cells was significantly increased after HGF induction under normal oxygen conditions ($P < 0.05$), whereas HLA-G1 inhibited the induction (Fig. 4I). Although HGF also induced cell invasion under hypoxia conditions ($P < 0.05$), HLA-G1 could not notably influence the cell invasion induced by HGF ($P > 0.05$). The effect of HLA-G1 on cell invasion of HTR-8/SVneo cells was consistent with the JAR cells.

4. Discussion

HLA-G expression level in extravillous trophoblasts is higher when the trophoblasts are close to the uterine spiral artery [26]. The specific characteristic of HLA-G plays an important role in protecting extravillous trophoblasts when they invade into the maternal blood vessels. For successful embryo implantation, it is necessary to establish contact between trophoblasts and the maternal uterus, and HLA-G is the key factor which can help trophoblasts invade into maternal decidua [27]. It has been reported that the expression of HLA-G has a close relationship with cleavage rate, which is an important index for assessing embryo quality in the process of *in vitro* fertilization [28–31]. Hamai discovered that HLA-G eliminates IL-2 inhibition of trophocyte proliferation and growth [32]. All of the previous research suggests that HLA-G plays an important role in embryogenesis and trophocyte proliferation

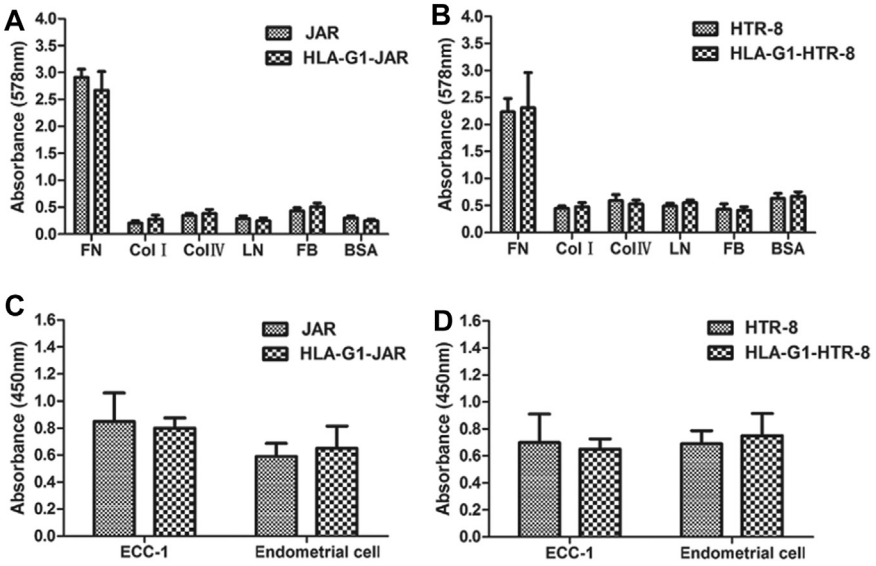

Fig. 3. Effect of HLA-G1 on cell adhesion. ECM/ECC-1/primary endometrial cells were packed in culture plates. JAR and HTR-8/SVneo cells were seeded in the wells. The adhered cells were dyed and the absorbance was measured. (A–B) The adhesive attraction between trophoblast cells and various components of the ECM. (C–D) endometrial cells. Data are represented as mean ± SD of three dependent experiments.

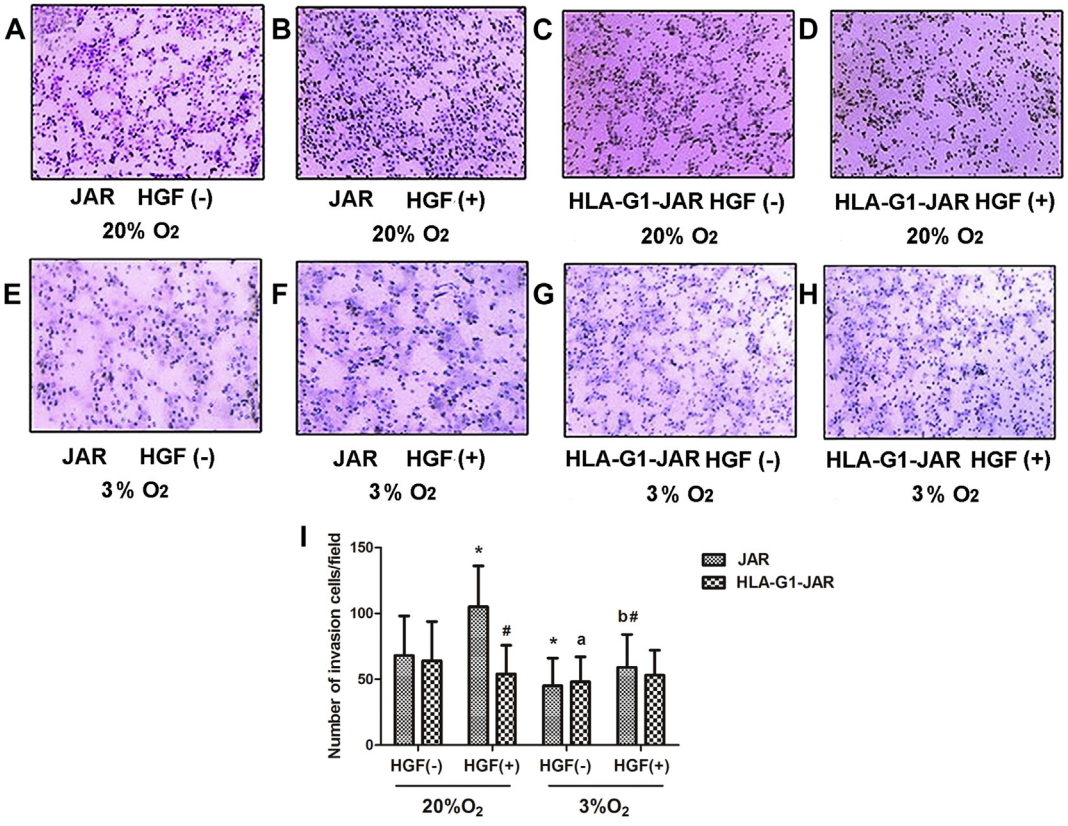

Fig. 4. Effect of HLA-G1 on cell invasion under different conditions. The invasion of JAR and HTR-8/SVneo cells under different conditions was detected by the transwell invasion chamber assay. (A–H) H&E staining of invasive cells with different treatments. (I) Quantitative description of invasive JAR cells under different conditions. $^{*}P < 0.05$ vs. JAR without HGF induction under normal oxygen condition; $^{\#}P < 0.05$ vs. JAR with HGF induction under normal oxygen condition; $^{a}P < 0.05$ vs. HLA-G1-JAR without HGF induction under normal oxygen condition; $^{b}P < 0.05$ vs. JAR without HGF induction under hypoxia condition. The image of H&E staining is at 200 × magnification. Data are represented as mean ± SD of three dependent experiments.

and growth, but there has been no systematic study on the relationship between HLA-G expression and trophocyte proliferation so far.

In this study, the relationship between HLA-G1 expression and trophocyte proliferation was detected, but no significant correlation was found. There are many cytokines that regulate cell growth *in vivo*. A previous study has found that HLA-G is a kind of signaling molecule involved in signal transduction [33]. We inferred that HLA-G1 may regulate trophocyte proliferation that is indirectly mediated by other molecules. To explore the exact impact of HLA-G1 on trophocyte proliferation, an *in vivo* test on the function of HLA-G1 should be carried out. Although there are eight subtypes of HLA-G, it is not clear whether other subtypes have the effect of regulating trophocyte proliferation.

HLA-G expression was decreased in the trophocytes of patients with preeclampsia, and the invasive ability of trophocytes was also decreased [34]. McCormick discovered that HLA-G can up-regulate the level of matrix metalloproteinase to facilitate trophocyte invasion [26]. To confirm this viewpoint, we explored the effect of HLA-G1 on trophocyte adhesion and invasion. Results showed that HLA-G1 could not influence the cell adhesion and invasion of trophocyte under conventional culture conditions. Trophocyte invasion is affected by a number of factors, such as hypoxia and HGF [35,36]. Further research found that HLA-G1 inhibited JAR and HTR-8/SVneo cell invasion induced by HGF under normal oxygen condition. While the exact mechanism remains unclear, the results indicated that HLA-G1 could regulate cell invasion, together with other factors in certain condition.

In conclusion, our study analyzed the influence of HLA-G1 on the proliferation, adhesion, and invasion of trophocytes comprehensively, and found that HLA-G1 was not an independent factor in regulating the proliferation, adhesion, and invasion of trophocytes. In addition, HLA-G1 inhibited JAR and HTR-8/SVneo cell invasion induced by HGF; this inhibition was weakened under hypoxic conditions. This finding emphasizes that embryo implantation is regulated by many factors and indicated that HLA-G1 may play an indirect effect in embryo implantation and formation of the placenta.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

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Transparency document

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References

[1] U.A. Kayisli, E.T. Korgun, G. Akkoyunlu, A. Arici, R. Demir, Expression of integrin alpha5 and integrin beta4 and their extracellular ligands fibronectin and laminin in human decidua during early pregnancy and its sex steroid-mediated regulation, Acta Histochem. 107 (2005) 173–185.
[2] K. Spiess, W.R. Teodoro, T.M. Zorn, Distribution of collagen types I, III, and V in pregnant mouse endometrium, Connect. Tissue Res. 48 (2007) 99–108.
[3] A. Makrigiannakis, M. Karamouti, P. Drakakis, D. Loutradis, A. Antsaklis, Fetomaternal immunotolerance, Am. J. Reprod. Immunol. 60 (2008) 482–496.
[4] P.C. Arck, K. Hecher, Fetomaternal immune cross-talk and its consequences for maternal and offspring's health, Nat. Med. 19 (2013) 548–556.
[5] S. Kovats, E.K. Main, C. Librach, M. Stubblebine, S.J. Fisher, R. DeMars, A class I antigen, HLA-G, expressed in human trophoblasts, Science 248 (1990) 220–223.
[6] S. Sakaguchi, K. Fukuma, K. Kuribayashi, T. Masuda, Organ-specific autoimmune diseases induced in mice by elimination of T cell subset. I. Evidence for the active participation of T cells in natural self-tolerance; deficit of a T cell subset as a possible cause of autoimmune disease, J. Exp. Med. 161 (1985) 72–87.
[7] S. Sakaguchi, N. Sakaguchi, M. Asano, M. Itoh, M. Toda, Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases, J. Immunol. 155 (1995) 1151–1164.
[8] S. Nardi Fda, R. Slowik, P.F. Wowk, J.S. da Silva, G.F. Gelmini, T.F. Michelon, J. Neumann, G. Bicalho Mda, Analysis of HLA-G polymorphisms in couples with implantation failure, Am. J. Reprod. Immunol. 68 (2012) 507–514.
[9] R. Rizzo, M. Vercammen, H. van de Velde, P.A. Horn, V. Rebmann, The importance of HLA-G expression in embryos, trophoblast cells, and embryonic stem cells, Cell. Mol. Life Sci. 68 (2011) 341–352.
[10] F. Puppo, M. Ghio, P. Contini, S. Brenci, D. Bignardi, G. Filaci, M. Scudeletti, F. Indiveri, Immunoregulatory role of soluble HLA molecules: a new skin for an old subject? Arch. Immunol. Ther. Exp. (Warsz.) 46 (1998) 157–160.
[11] M. Cecati, S.R. Giannubilo, M. Emanuelli, A.L. Tranquilli, F. Saccucci, HLA-G and pregnancy adverse outcomes, Med. Hypotheses 76 (2011) 782–784.
[12] R. Hackmon, M. Hallak, M. Krup, D. Weitzman, E. Sheiner, B. Kaplan, Y. Weinstein, HLA-G antigen and parturition: maternal serum, fetal serum and amniotic fluid levels during pregnancy, Fetal Diagn. Ther. 19 (2004) 404–409.
[13] S.M. Yie, R.N. Taylor, C. Librach, Low plasma HLA-G protein concentrations in early gestation indicate the development of preeclampsia later in pregnancy, Am. J. Obstet. Gynecol. 193 (2005) 204–208.
[14] A. Steinborn, T. Varkonyi, A. Scharf, F. Bahlmann, A. Klee, C. Sohn, Early detection of decreased soluble HLA-G levels in the maternal circulation predicts the occurrence of preeclampsia and intrauterine growth retardation during further course of pregnancy, Am. J. Reprod. Immunol. 57 (2007) 277–286.
[15] A.M. Borzychowski, I.L. Sargent, C.W. Redman, Inflammation and pre-eclampsia, Semin. Fetal Neonatal Med. 11 (2006) 309–316.
[16] K.A. Pfeiffer, V. Rebmann, M. Passler, K. van der Ven, H. van der Ven, D. Krebs, H. Grosse-Wilde, Soluble HLA levels in early pregnancy after in vitro fertilization, Hum. Immunol. 61 (2000) 559–564.
[17] J. LeMaoult, I. Krawice-Radanne, J. Dausset, E.D. Carosella, HLA-G1-expressing antigen-presenting cells induce immunosuppressive CD4+ T cells, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 7064–7069.
[18] J.M. Lozano, R. González, J.M. Kindelán, N. Rouas-Freiss, R. Caballos, J. Dausset, E.D. Carosella, J. Peña, Monocytes and T lymphocytes in HIV-1-positive patients express HLA-G molecule, Aids 16 (2002) 347–351.
[19] N. Rouas-Freiss, P. Moreau, C. Menier, J. LeMaoult, E.D. Carosella, Expression of tolerogenic HLA-g molecules in cancer prevents antitumor responses, Semin. Cancer Biol. (2007) 413–421. Elsevier.
[20] E.D. Carosella, B. Favier, N. Rouas-Freiss, P. Moreau, J. LeMaoult, Beyond the increasing complexity of the immunomodulatory HLA-G molecule, Blood 111 (2008) 4862–4870.
[21] H. Wiendl, U. Feger, M. Mittelbronn, C. Jack, B. Schreiner, C. Stadelmann, J. Antel, W. Brueck, R. Meyermann, A. Bar-Or, Expression of the immune-tolerogenic major histocompatibility molecule HLA-G in multiple sclerosis: implications for CNS immunity, Brain 128 (2005) 2689–2704.
[22] E. Fainardi, R. Rizzo, L. Melchiorri, M. Castellazzi, E. Paolino, M. Tola, E. Granieri, O. Baricordi, Intrathecal synthesis of soluble HLA-G and HLA-I molecules are reciprocally associated to clinical and MRI activity in patients with multiple sclerosis, Mult. Scler. 12 (2006) 2–12.
[23] J. Kwak-Kim, A. Gilman-Sachs, Review article: clinical implication of natural killer cells and reproduction, Am. J. Reprod. Immunol. 59 (2008) 388–400.
[24] M. Ponte, C. Cantoni, R. Biassoni, A. Tradori-Cappai, G. Bentivoglio, C. Vitale, S. Bertone, A. Moretta, L. Moretta, M.C. Mingari, Inhibitory receptors sensing HLA-G1 molecules in pregnancy: decidua-associated natural killer cells express LIR-1 and CD94/NKG2A and acquire p49, an HLA-G1-specific receptor, Proc. Natl. Acad. Sci. 96 (1999) 5674–5679.
[25] K. Diedrich, B.C. Fauser, P. Devroey, G. Griesinger, The role of the endometrium and embryo in human implantation, Hum. Reprod. Update 13 (2007) 365–377.
[26] J. McCormick, G.S. Whitley, P. Le Bouteiller, J.E. Cartwright, Soluble HLA-G regulates motility and invasion of the trophoblast-derived cell line SGHPL-4, Hum. Reprod. 24 (2009) 1339–1345.
[27] M.T. McMaster, C.L. Librach, Y. Zhou, K.-H. Lim, M.J. Janatpour, R. DeMars, S. Kovats, C. Damsky, S.J. Fisher, Human placental HLA-G expression is restricted to differentiated cytotrophoblasts, J. Immunol. 154 (1995) 3771–3778.
[28] C.M. Warner, P.W. Lampton, J.A. Newmark, J. Cohen, Symposium: innovative techniques in human embryo viability assessment. Soluble human leukocyte antigen-G and pregnancy success, Reprod. Biomed. Online 17 (2008) 470–485.
[29] A. Jurisicova, R.F. Casper, N.J. MacLusky, G.B. Mills, C.L. Librach, HLA-G expression during preimplantation human embryo development, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 161–165.

[30] S.-m. Yie, H. Balakier, G. Motamedi, C.L. Librach, Secretion of human leukocyte antigen-G by human embryos is associated with a higher in vitro fertilization pregnancy rate, Fertil. Steril. 83 (2005) 30–36.
[31] R.G. Roussev, C.B. Coulam, HLA-G and its role in implantation (review), J. Assist. Reprod. Genet. 24 (2007) 288–295.
[32] Y. Hamai, T. Fujii, T. Yamashita, A. Miki, H. Hyodo, S. Kozuma, D.E. Geraghty, Y. Taketani, The expression of human leukocyte antigen-G on trophoblasts abolishes the growth-suppressing effect of interleukin-2 towards them, Am. J. Reprod. Immunol. 41 (1999) 153–158.
[33] M. Comiskey, K.E. Domino, C.M. Warner, HLA-G is found in lipid rafts and can act as a signaling molecule, Hum. Immunol. 68 (2007) 1–11.
[34] H. Li, J. Dakour, L.J. Guilbert, B. Winkler-Lowen, F. Lyall, D.W. Morrish, PL74, a novel member of the transforming growth factor-β superfamily, is overexpressed in preeclampsia and causes apoptosis in trophoblast cells, J. Clin. Endocrinol. Metab. 90 (2005) 3045–3053.
[35] O. Genbacev, Y. Zhou, J.W. Ludlow, S.J. Fisher, Regulation of human placental development by oxygen tension, Science 277 (1997) 1669–1672.
[36] J.E. Cartwright, D.P. Holden, G.S. Whitley, Hepatocyte growth factor regulates human trophoblast motility and invasion: a role for nitric oxide, Br. J. Pharmacol. 128 (1999) 181–189.