Biochemical and Biophysical Research Communications xxx (2015) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

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

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ARTICLE INFO

Article history:
Received 8 January 2015
Available online xxx

Keywords:
HLA-G1
Trophoblast cells
Embryo implantation
Proliferation
Adhesion
Invasion

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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http://dx.doi.org/10.1016/j.bbrc.2015.01.085
0006-291X/

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

Please cite this article in press as: F. Jiang, et al., Role of HLA-G1 in trophoblast cell proliferation, adhesion and invasion, Biochemical and Biophysical Research Communications (2015), http://dx.doi.org/10.1016/j.bbrc.2015.01.085