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Upregulation of HAb18G/CD147 in activated human umbilical vein endothelial cells enhances the angiogenesis

Yanke Chen [a,1], Hongxin Zhang [b,1], Xingchun Gou [a], Yohei Horikawa [c], Jinliang Xing [a,*], Zhinan Chen [a,*]

[a] Department of Cell Biology and Cell Engineering Research Center and State Key Laboratory of Cancer Biology, Fourth Military Medical University, 17 West Changle Street, Xi'an 710032, China
[b] Department of Interventional Radiology, Tangdu Hospital, Fourth Military Medical University, Xi'an 710038, China
[c] Department of Urology, Akita University School of Medicine, Akita, Japan

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ABSTRACT

Previous studies demonstrated that CD147 molecule, highly expressed on the surface of various malignant tumor cells, significantly correlated with the malignancy of these cancers; however, the role of HAb18G/CD147 in endothelial cells has yet to be established. In this study, we found that the expression of HAb18G/CD147 was significantly upregulated in activated HUVECs. The inhibition of HAb18G/CD147 expression by specific siRNA led to significantly decreased angiogenesis in vitro. Our data indicate that HAb18G/CD147 may regulate angiogenesis via several mechanisms including proliferation, survival, migration, MMPs secretion, and PI3K/Akt activation. Our findings for the first time suggest that upregulation of HAb18G/CD147 in activated HUVECs might play an important role in angiogenesis.

1. Introduction

HAb18G /CD147, which was found on the surface of human hepatoma cells, is a highly glycosylated transmembrane protein that contains two extracellular immunoglobulin domains (C and V domains) and belongs to the immunoglobulin superfamily [1]. Various independent laboratories have discovered the CD147 protein in different origins of human cells and tissues, designating it extracellular matrix metalloproteinase inducer (EMMPRIN) [2], basigin [3] or M6 antigen [4]. Several proteins with high levels of homology to HAb18G/CD147, i.e. neurothelin, HT7, OX47, and gp42, have also been characterized in other species [5].

Previous studies demonstrated that CD147 molecule is highly expressed on the surface of various malignant tumor cells, including cancers of liver [6], skin [7], lung [8], breast [9], bladder [10], and brain [11]. Elevated CD147 expression is significantly correlated with the malignancy of these cancers. The biological implication of increased CD147 in tumor cells has been investigated by in vitro studies using recombinant CD147 or native CD147 purified from tumor cells, indicating that CD147 mainly functions as an inducer of MMPs production in tumor local environment [12,13]. CD147-positive tumor cells stimulate adjacent fibroblast cells to secrete MMPs (mainly including MMP-2 and MMP-9) and thus promote tumor invasion and metastasis [14].

Except for tumor cells, CD147 is also expressed at varying levels in many other cell types, including activated T cells [15], differentiated macrophage [16], epithelial and endothelial cells [17], and normal human keratinocytes [18]. The expression of CD147 in non-tumor tissues suggests that this molecule may be also involved in other

* Corresponding authors. Tel.: +86 29 84774573; fax: +86 29 83226399 (J. Xing), tel.: +86 29 84774547; fax: +86 29 83293906 (Z. Chen).
E-mail addresses: xingjl@fmmu.edu.cn (J. Xing), znchen@fmmu.edu.cn (Z. Chen).
[1] Yanke Chen and Hongxin Zhang equally contributed to this work.

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physiological and/or pathological processes that may be associated with increased MMPs expression. For example, its presence in the epidermis and several embryonic epithelia suggests that CD147 may participate in epithelial-mesenchymal interactions, leading to changes in tissue architecture during embryonic development [19] and wound healing [20]. Also, CD147 on the surface of activated lymphocytes and monocytes may result in elevated MMPs levels and therefore contribute to progression of chronic inflammation [21,22].

Most recently, the biological activity of CD147 has been linked to the tumor angiogenesis. Tumors depend upon angiogenesis for growth and the development of metastases. Tumor angiogenesis is a complex and multi-step process requiring the sequential activation of various factors. Tang et al. [23] found that CD147 stimulates production of VEGF in tumor cells, therefore indicating its involvement in regulating tumor angiogenesis. Millimaggi et al. [24] reported that CD147 is expressed in microvesicles derived from epithelial ovarian cancer cells and CD147-positive vesicles promote an angiogenic phenotype in endothelial cells in vitro. In studies mentioned above, the study of CD147 in tumor angiogenesis was mainly focused on its tumor cell-derived expression. A previous report has shown that CD147 is also expressed in HUVECs [25], suggesting that this molecule may play a role in angiogenic function of HUVECs. However, no data are available on the functional role of HAb18G/CD147 in endothelial cells.

A recent study by Tang et al. [12] reported a positive feedback regulation model of CD147 expression in which tumor cell-associated CD147 stimulates its own expression in tumor stroma, consequently contributing to tumor angiogenesis, tumor growth, and metastasis. Therefore, we hypothesize that HAb18G/CD147 expression in endothelial cells around tumor cells can be up-regulated and thus contribute to the tumor angiogenesis. In the present study, we investigated the expression of HAb18G/CD147 in activated HUVECs and its effect on angiogenic phenotype of HUVECs. To the best of our knowledge, this is the first study to examine the functional role of HAb18G/CD147 in HUVECs.

2. Materials and methods

2.1. Cell cultures

HUVECs was isolated and cultured as previously described [26]. In brief, human umbilical cord veins were digested with collagenase (Roche Diagnostics). Cells were routinely cultured in flasks coated with 0.2% gelatin in endothelial basal medium (EBM-2) (Lonza, Walkersville, MD) supplemented with 10% fetal bovine serum (FBS). To activate the quiescent HUVECs, cells were then grown in endothelial cell growth medium (EGM-2) (Lonza, Walkersville, MD) which is composed of EBM-2 and supplements, such as FBS, and growth factors including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF) and insulin-like growth factor-I (IGF-I). For all experiments, activated HUVECs from the second to eighth passages were used.

2.2. Flow cytometry

Quiescent and activated HUVECs were harvested and washed once with PBS, and then incubated at 4 °C for 30 min with 1 μg/ml of RPE-conjugated anti-CD147 antibody (AbD Serotec, UK) in PBS containing 0.5% BSA. After washing three times with PBS, cell samples were fixed in 4% paraformaldehyde for at least 15 min at room temperature and analyzed with FACSCalibur flow cytometer and CellQuest software (Becton Dickinson). Omission of the antibody was used as control. The mean fluorescence intensity from three independent experiments was calculated and presented as mean ± SD.

2.3. Immunofluorescence staining

The quiescent and activated HUVECs were first grown to confluence on glass coverslips coated with gelatin, respectively. Then, cells on coverslips were washed once with PBS and fixed in 4% paraformaldehyde for 15 minutes, followed by incubation with anti-human HAb18G/CD147 monoclonal antibody (2 μg/ml, prepared in our laboratory) for 30 min at room temperature. After washing three times with PBS, cell samples were next incubated with FITC-conjugated goat anti-mouse IgG (1:2000) (Pierce, Rockford, IL). Finally, cells were observed using confocal microscope (Bio-Rad MRC1024, Bio-Rad Inc.).

2.4. Small interfering RNA (siRNA)

The activated HUVECs growing up to 70–80% confluency were transfected with 50 pM siRNA that specifically targets HAb18G/CD147 gene or 50 pM nonsilencing control siRNA (non-specific scrambled siRNA) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Transfected cells were cultured for 24–48 h and then used for further functional analysis. All siRNA duplexes were synthesized by Ambion Inc. (Austin, TX). The sequences of siRNA duplex targeting HAb18G/CD147 are as following: 5′-GUUCUUCGUGAGUUCCUCdTdT-3′ and 3′-dTdTCAAGAAGCACUCAAGGAG-5′.

2.5. Western blot analysis

Cell samples were lysed with RIPA buffer (Beyotime Inc., NanTong, China) and protein concentrations were measured using the micro BCA Assay (Pierce, Rockford, IL). The equal amounts (10 μg) of total protein was separated on 12% SDS–PAGE, and then transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA). The membranes were subsequently immunoblotted with the appropriate primary antibody. The following primary antibodies were used in this study: anti-HAb18G/CD147 antibody, anti-FAK and anti-phosphorylated FAK (p-FAK) from Sigma, anti Akt and anti-phosphorylated Akt (ser 473) antibody from Cell Signaling Technology (Beverly, MA). After extensive washings, the membranes were incubated with a secondary horseradish peroxidase-conjugated goat anti-mouse antibody (Pierce, Rockford, IL). Signals were detected using an ECL kit (Pierce, Rockford, IL) according to the manufacturer's instructions.