Cancer Letters 278 (2009) 113–121

Contents lists available at ScienceDirect

Cancer Letters

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

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

ARTICLE INFO

Article history:
Received 28 October 2008
Received in revised form 24 December 2008
Accepted 5 January 2009

Keywords:
Angiogenesis
HAb18G/CD147
Human umbilical vein endothelial cells

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.

0304-3835/$ - see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2009.01.004

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.

2.6. In vitro Matrigel tube formation assay

To prepare Matrigel-coated plates, Matrigel matrix (Becton Dickinson) was added evenly into a 96-well plate (50 μl/well), and incubated for 40 min at 37 °C. After culturing for 36 h, activated HUVECs transfected with HAb18G/CD147 siRNA and control siRNA were trypsin-released and diluted with EGM-2 to a final concentration of 1×10^5 ml^{-1}, and then seeded onto Matrigel-coated wells (1×10^4 cells/well). Capillary tube formation was observed at 1 h, 2 h, 4 h and 6 h after cell seeding with a phase-contrast microscope (Olympus CKX41). Tube formation ability was quantified by counting the number of branches per field. Results, each from four randomly selected fields, are expressed as mean ± SD of three independent experiments.

2.7. MTT assay

After transfection with HAb18G/CD147 siRNA and control siRNA for 24 h, the activated HUVECs were plated in 96-well plates (1×10^4 cells/well) and continued to culture in EGM-2 medium. The in vitro proliferation ability of HUVECs was measured over 72 h using the methyl thiazolyl tetrazolium (MTT) (Sigma) assay according to the manufacturer's instruction.

2.8. TUNEL assay

After transfection with siRNA for 24 h, the activated HUVECs were plated on glass coverslips coated with gelatin. Then, apoptosis was induced by culturing cells in EBM-2 medium containing 1% FBS for 12 h, 24 h, and 48 h. TUNEL staining was performed using In Situ Cell Death Detection Kit (Roche Diagnostics) per manufacturer's instructions. DAPI counterstain was used to quantify cells with intact nuclei. The percentage of TUNEL-positive cells was calculated by dividing the number of TUNEL-positive cells by the number of DAPI-positive nuclei at ×100 magnification for four fields for each sample.

2.9. In vitro invasion assay

The migration ability of HUVECs was measured by in vitro invasion assay using modified Boyden chambers with polycarbonate filters (pore size, 8 μm). Before using, Matrigel (Becton Dickinson) was coated on the upper side of filters. After transfection with siRNA for 24 h, activated HUVECs were detached, washed in PBS, resuspended in serum-free EBM-2 medium containing 1% BSA, and then added to the upper compartment of chamber at a total number of 5×10^3 cells per chamber. The lower compartment of chambers was filled with serum-free EBM-2 medium containing 5% BSA, 10 ng/ml human VEGF and 10 ng/ml human bFGF. After incubation for 12 h, 24 h and 36 h, the number of cells migrating through the filter was counted by Haematoxylin and Eosin staining and plotted as the mean number of migrating cells per optic field in three independent experiments.

2.10. Gelatin zymography

After transfection with siRNA for 24 h, activated HUVECs were grown in 96-well plate coated with Matrigel in EGM-2 medium for 12 h, and then incubated in serum-free EBM-2 for 12 h. Consequently, serum-free conditioned medium was harvested. Secretion and activation level of MMP-2 and MMP-9 in conditioned medium were detected using gelatin zymography as previously described [27].

2.11. Statistical analysis

All statistical analyses were performed using the SPSS 13.0 statistical software package (SPSS, Chicago, IL). Statistical significance of the difference was determined by the Student's *t*-test. All *P* values were based on two-sided tests. A probability level of 0.05 was used as the criterion for statistical significance. Mean (standard deviation, SD) values of three independent experiments are presented for all samples.

3. Results

3.1. HAb18G/CD147 was upregulated in activated HUVECs

The expression level of HAb18G/CD147 was evaluated in quiescent and activated HUVECs. Flow cytometry analysis showed that the HAb18G/CD147 was significantly upregulated in activated HUVECs, compared with quiescent cells. The mean fluorescence intensities were 321 ± 7.8 in activated HUVECs and 106 ± 5.1 in quiescent cells, respectively (Fig. 1A). Similar result was also observed in Western blot analysis, indicating that the expression of HAb18G/CD147 is notably higher in activated HUVECs than quiescent cells (Fig. 1B). Immunofluorescence staining analysis demonstrated that, compared with the quiescent cells, the HAb18G/CD147 was upregulated in activated HUVECs and this molecule mainly located on the surface of both quiescent and activated HUVECs (Fig. 1C).

3.2. HAb18G/CD147 siRNA leads to significantly decreased angiogenesis in activated HUVECs

To investigate the role of HAb18G/CD147 in the angiogenesis of activated HUVECs, we transfected HAb18G/CD147 siRNA into activated HUVECs and then evaluated the ability of in vitro tube formation of HUVECs on Matrigel. Western blot analysis showed that the expression of HAb18G/CD147 was significantly decreased in activated HUVECs transfected with HAb18G/CD147 siRNA, compared with those transfected with control siRNA (Fig. 2A). In vitro tube formation evaluation indicated that the branches and tubes from activated HUVECs transfected with HAb18G/CD147 siRNA were much less formed compared with those transfected with control siRNA at 2 h, 4 h and 6 h after activated HUVECs were seeded on Matrigel ($P < 0.05$, $P < 0.01$ and $P < 0.01$, respectively) (Fig. 2B and C). Our data indicate that the ability of tube formation on Matrigel is significantly decreased in activated HUVECs with down-regulated expression of HAb18G/CD147.

3.3. HAb18G/CD147 siRNA reduces the ability of in vitro proliferation, migration and MMPs secretion in activated HUVECs

The proliferation kinetics of activated HUVECs was shown in Fig. 3A. We found that the ability of in vitro proliferation was considerably inhibited in activated HUVECs transfected with HAb18G/CD147 siRNA, compared with those transfected with control siRNA. In vitro invasion assay demonstrated that the number of cells migrating through the filter was much less in activated HUVECs transfected with HAb18G/CD147 siRNA than those transfected with control siRNA at all time points studied (all $P < 0.05$) (Fig. 3B). Furthermore, gelatin zymography analysis showed that the secretion and activation of MMP-2 and MMP-9 were significantly decreased in activated HUVECs transfected with HAb18G/CD147 siRNA, compared with those transfected with control siRNA (Fig. 3C). These results indicate that the down-regulation of HAb18G/CD147 expression may attenuate the ability of in vitro proliferation, migration and MMPs secretion in activated HUVECs.

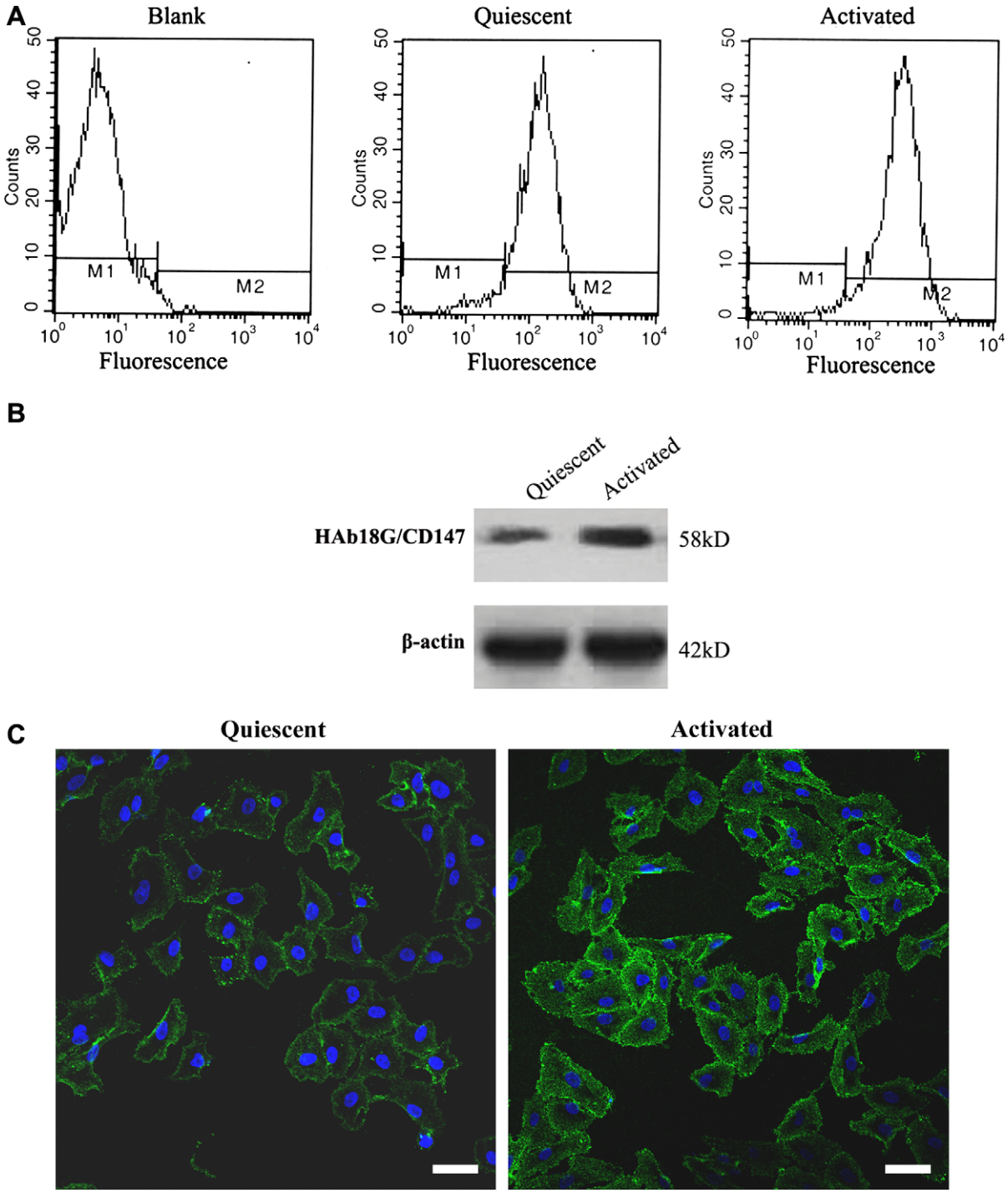

Fig. 1. Expression and location of HAb18G/CD147 in HUVECs. (A and B) Flow cytometry and Western blot analysis of HAb18 G/CD147 expression in quiescent and activated HUVECs. (C) Immunofluorescence staining of quiescent (left) and activated (right) HUVECs with anti-HAb18G/CD147 monoclonal antibody. Scale bar, 50 μm.

3.4. HAb18G/CD147 protects activated HUVECs from apoptosis in response to growth-factor deprivation

TUNEL and DAPI staining was used to assess the apoptosis of activated HUVECs induced by growth-factor deprivation. As shown in Fig. 4A and B, we found that activated HUVECs transfected with HAb18G/CD147 siRNA had a significantly higher apoptosis rate than those transfected with control siRNA at 12 h, 24 h and 48 h after growth-factor deprivation ($P < 0.05$, $P < 0.001$ and $P < 0.001$, respectively). These data suggest that the down-regulation of HAb18G/CD147 expression may predispose activated HUVECs to apoptosis.

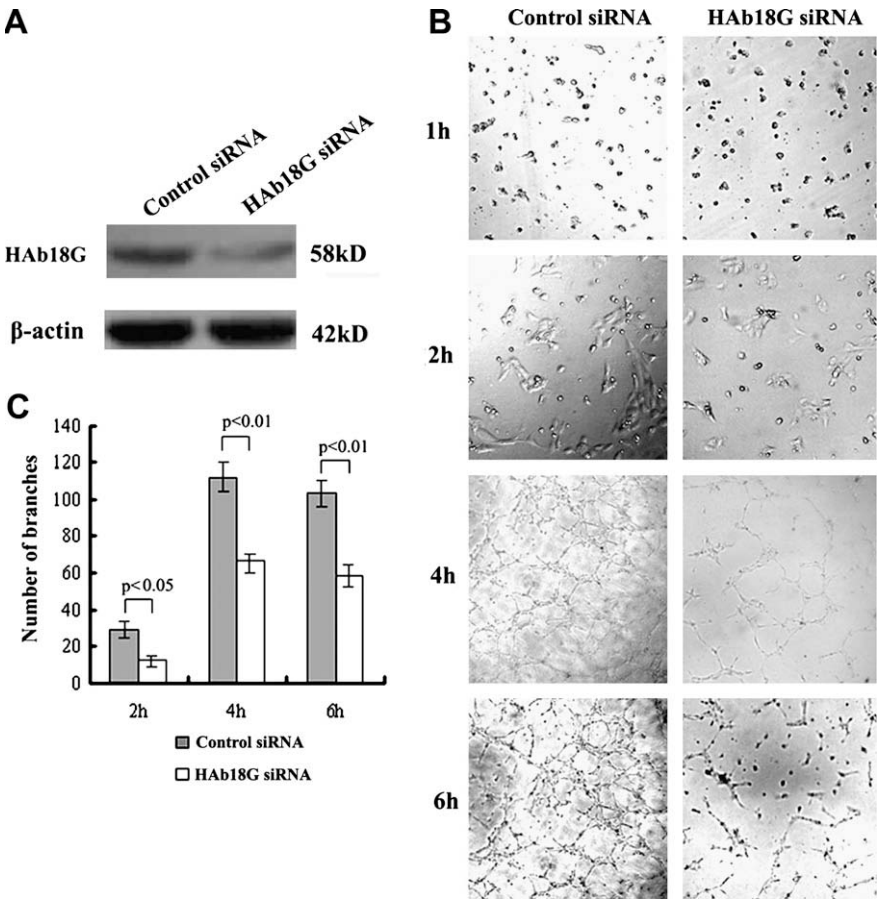

Fig. 2. Inhibition of angiogenesis in activated HUVECs by HAb18G/CD147 siRNA. (A) Western blot analysis of HAb18G/CD147 expression in activated HUVECs 48 h after transfection with HAb18G/CD147 siRNA and control siRNA. (B and C) In vitro capillary tube formation of activated HUVECs on Matrigel. Activated HUVECs were transfected with HAb18G/CD147 siRNA and control siRNA and further cultured for 36 h, then seeded on Matrigel. Images were acquired at 1 h, 2 h, 4 h, and 6 h after seeding using an inverted microscope (Olympus CKX41) fitted with a 10× phase-contrast objective lens. (B) Representative photomicrographs of capillary-like structures from activated HUVECs on Matrigel. Scale bars, 100 μm. (C) Semi-quantitative assessment of pseudotube formation was performed by determining the number of branches per field. Results, each based on four randomly selected fields, are expressed as mean ± SD of three independent experiments. Statistical significance was determined by Student's *t*-test.

3.5. HAb18G/CD147 enhances angiogenesis of activated HUVECs via the PI3K-AKT pathway

To elucidate the possible signaling pathway involved in the enhancement of angiogenesis by HAb18G/CD147, Western blot analysis was performed to examine the expression level of HAb18G/CD147, phosphorylated FAK, FAK, phosphorylated Akt and Akt in activated HUVECs transfected with HAb18G/CD147 siRNA and control siRNA. We found that the down-regulation of HAb18G/CD147 resulted in the significant decrease of phosphorylated FAK and phosphorylated Akt level. However, expression of total FAK and Akt was not affected (Fig. 5). These data suggest that the PI3K-Akt signaling pathway might be involved in the enhancement of angiogenesis by HAb18G/CD147 in activated HUVECs.

4. Discussion

In our study, we evaluated the expression and angiogenic function of HAb18G/CD147 in activated HUVECs. We found that HAb18G/CD147 was significantly upregulated in activated HUVECs, compared with quiescent cells. More importantly, our data demonstrated that the upregulated HAb18G/CD147 enhances angiogenesis through promoting in vitro proliferation, migration and MMPs secretion of activated HUVECs and protecting these cells from apoptosis. In addition, the enhancement of angiogenesis was noted to possibly involve the PI3K-Akt signaling pathway.

It has been extensively reported that HAb18G/CD147 is enriched on the surface of various tumor cells and stimulates production of several matrix metalloproteinases [28,29]. Recently, more research attention has been paid on the expression and function of CD147 in other tissues including the developing retina [30], blood–brain barrier [31], nerve system [32], thymus [33], epithelium [34]. However, the knowledge about the expression of HAb18G/CD147 in HUVECs is very limited. To date, a single report by Mutin et al. [25] demonstrated that CD147 is a surface molecular marker on HUVECs with a moderate expression level under resting condition, they also found that CD147 could be 1.5-fold upregulated in IL-1 activated HUVECs but not in TNF-α activated HUVECs. Menashi et al. [35] reported that the expression of CD147 is upregulated by epidermal growth factor (EGF) in transformed human breast epithelial cells. In the present study, the HUVECs were activated with a mixture of proangiogenic growth

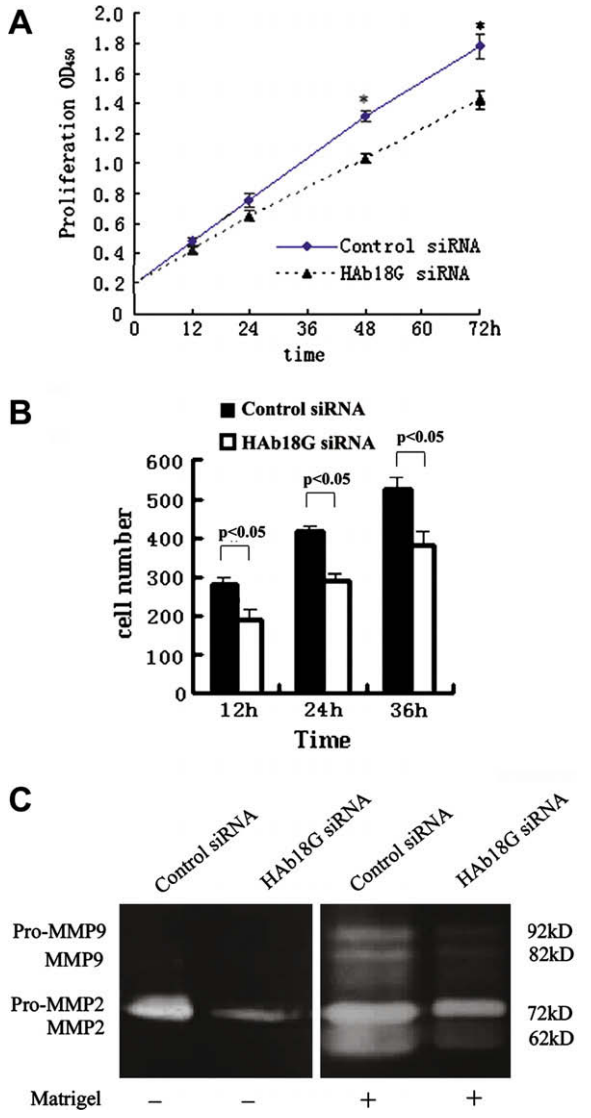

Fig. 3. Evaluation on the ability of in vitro proliferation, migration and MMPs secretion in activated HUVECs. (A) In vitro proliferation ability was measured by MTT assay over 3 days in activated HUVECs 24 h after transfection with HAb18G/CD147 siRNA and control siRNA. Data are presented as absorbance at optical density (OD) 490 nm (mean ± SD of three replicate experiments). $^{*}P < 0.05$, compared to the control level. (B) Migration ability of activated HUVECs was examined by in vitro invasion assay 24 h after transfection with HAb18G/CD147 siRNA and control siRNA. Cells were first plated on the upper compartment of Chambers. After incubation for 12 h, 24 h, and 36 h, the number of cells migrating through the filter was counted and plotted as the mean number of migrating cells per optic field in three independent experiments. Columns stand for mean ± SD. Statistical significance was determined by Student's *t*-test. (C) MMPs secretion was analyzed by Gelatin zymography in activated HUVECs 48 h after transfection with HAb18G/CD147 siRNA and control siRNA.

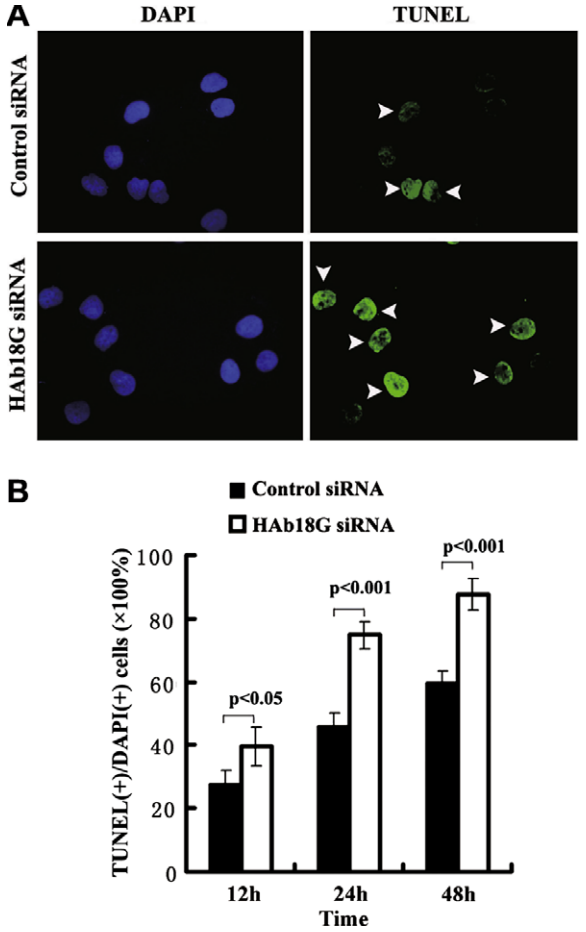

Fig. 4. Apoptosis analysis of activated HUVECs in response to growth-factor deprivation. (A) TUNEL and DAPI staining was used to assess the apoptosis of activated HUVECs induced by growth-factor deprivation for 12 h, 24 h and 48 h after cells was transfected with HAb18G/CD147 siRNA or control siRNA and cultured for 24 h. Representative photomicrographs of staining from activated HUVECs were exhibited. Arrowheads indicate the apoptosis cells. (B) Semi-quantitative evaluation of apoptosis was performed by determining the rate of TUNEL-positive cells/DAPI-positive cells in three independent experiments. Data, each based on four randomly selected fields, are expressed as mean ± SD. Statistical significance was determined by Student's *t*-test.

factors, including VEGF, bFGF, EGF and IGF. We obtained very similar results, indicating that expression of HAb18G/CD147 was significantly upregulated in activated HUVECs, compared with quiescent cells. Nevertheless, the molecular mechanisms involved in the upregulation of HAb18G/CD147 still need to be further elucidated.

The upregulation of HAb18G/CD147 in activated HUVECs has led us to hypothesize that this molecular may play an important role in angiogenesis. Thus, we investigated the effect of HAb18G/CD147 expression on the angiogenic phenotype of activated HUVECs. Our data indicated that HAb18G/CD147 siRNA could efficiently down-regulate the expression of this molecular in activated HUVEC, which is consistent with previous report from hepatocellular carcinoma cells [28]. Further, the down-regulated HAb18G/CD147 was demonstrated to considerably inhibit angiogenesis of activated HUVECs. To the best of our knowledge, our study is the first report to explore the angiogenic implication of HAb18G/CD147 in HUVECs. In previous studies, Tang et al. [23] first demonstrated in both in vitro and in vivo experiments that tumor cell-associated CD147 is involved in the tumor angiogenesis by

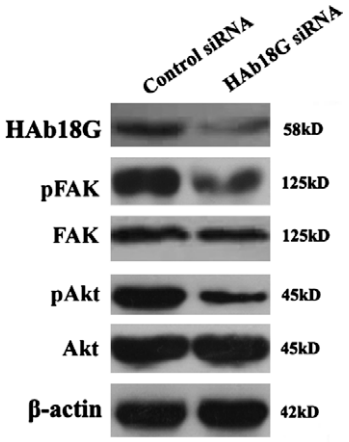

Fig. 5. Analysis of signaling pathway involved in the enhancement of angiogenesis by HAb18G/CD147 in activated HUVECs. Western blot was performed to examine the expression level of HAb18G/CD147, phosphorylated FAK, FAK, phosphorylated Akt and Akt in activated HUVECs transfected with HAb18G/CD147 siRNA and control siRNA. The expression of β-actin was used as internal control. All figures are a representation of three trials.

inducing VEGF and MMPs production. In clinical tissues, Zheng et al. [36] and Zhang et al. [37] found that the upregulated CD147 contributes to angiogenesis of gastric carcinoma and hepatocellular carcinoma, respectively. In addition, it has been reported that vesicles shed by ovarian cancer cells may induce proangiogenic activities of HUVECs by a CD147-mediated mechanism [24]. These findings strongly support the idea that except as a MMPs inducer, HAb18G/CD147 is still an important angiogenesis enhancer. Our study suggests a novel mechanism of tumor angiogenesis in which vesicular endothelial cells were activated by various tumor cell-derived factors to express high level of HAb18G/CD147, thus contribute to the enhancement of angiogenesis.

We also explored the functional mechanism by which HAb18G/CD147 enhances angiogenesis in activated HUVECs. We found that the down-regulated HAb18G/CD147 inhibits in vitro proliferation, migration and MMPs secretion of activated HUVECs. A similar result has been previously reported, demonstrating that HAb18G/CD147 knockdown resulted in the decreased proliferation and migration of Jurkat cells [38]. Caudroy et al. [39] have reported that tumor-derived CD147 induces the secretion of MMP-2 in HUVECs. Haseneen et al. [40] demonstrated that upregulation of CD147 involves in the activation of MMP-2 release in lung endothelium. However, these studies did not find the change of MMP-9 secretion, inconsistent with our finding that upregulated HAb18G/CD147 significantly induces the secretion of MMP-9 in HUVECs. The most possible reason to explain this difference is that two-dimension culturing was used in studies mentioned above instead of three-dimension (3D) culturing with Matrigel in our study. The 3D culturing is preferred because of functionally more similar to tissues in the human. In addition, Intasai et al. [41] revealed that CD147 induces apoptosis in U973 cells partially by involving a caspase-dependent pathway. In the present study, we found the opposite results, indicating that the down-regulated HAb18G/CD147 increases the apoptosis susceptibility of activated HUVEC to growth-factor deprivation. Baba et al. [42] also reported that the CD147 inhibition induces cell death specific to cancer cells that depend on glycolysis for their energy production. The explanation for the functional differences of HAb18G/CD147 among different types of cells needs to be further explored in future study.

Furthermore, we investigated the possible signaling molecules involved in the enhancement of angiogenesis by HAb18G/CD147. Our data confirmed that the down-regulated HAb18G/CD147 results in the decreased level of phosphorylated FAK, phosphorylated Akt, suggesting that these molecules-associated signaling pathway, especially PI3K-Akt pathway, might be involved in the function of HAb18G/CD147 in activated HUVECs. In previous studies, a series of evidence showed that PI3K-Akt pathway is one of most important signaling pathway in angiogenesis [43,44]. This pathway regulates a wide spectrum of cellular processes, including cell proliferation, survival, growth, and motility [45,46]. A previous study indicated that tumor-derived HAb18G/CD147 could lead to the activation of PI3K-Akt pathway in tumor cells [47]. Our study for the first time indicated that the similar signaling pathway might also exist in activated HUVECs. However, in most cases, the repertoire of upstream mediators and the downstream targets of this pathway remain unclear. The phosphorylated FAK has been reported to be a common activator of this pathway [48,49]. Our data also revealed a change in phosphorylated FAK level, suggesting that FAK might be one of the upstream mediators of PI3K-Akt pathway in activated HUVECs. Certainly, our preliminary data cannot rule out the possibility that other important signaling molecules involves in the function of HAb18G/CD147 in activated HUVECs.

In conclusion, our study for the first time provides the evidence that HAb18G/CD147 is significantly upregulated in activated HUVECs and the up-regulation of this molecule strongly enhances the angiogenic phenotype of activated HUVECs. These findings added new insights to the angiogenesis mechanism driven by HAb18G/CD147 in HUVECs and further highlight the importance of this molecule in tumor angiogenesis.

Acknowledgements

This work was supported by Grants 2006CB708504 and 2009CB521704 from the National Basic Research Program of China and Grant 30530720 from National Natural Science Foundation of China.

References

[1] Z.N. Chen, Z. Yang, L. Mi, J.L. Jiang, X.N. Guo, Analysis on the structure and function of hepatoma transfer-associated factor HAb18G, J. Cell Mol. Immunol. 15 (1999) 34.
[2] C. Biswas, Y. Zhang, R. DeCastro, H. Guo, T. Nakamura, H. Kataoka, K. Nabeshima, The human tumor cell-derived collagenase stimulatory factor (renamed EMMPRIN) is a member of the immunoglobulin superfamily, Cancer Res. 55 (1995) 434–439.
[3] T. Kanekura, T. Miyauchi, M. Tashiro, T. Muramatsu, Basigin, a new member of the immunoglobulin superfamily: genes in different

mammalian species, glycosylation changes in the molecule from adult organs and possible variation in the N-terminal sequences, Cell Struct. Funct. 16 (1991) 23–30.
[4] W. Kasinrerk, E. Fiebiger, I. Stefanova, T. Baumruker, W. Knapp, H. Stockinger, Human leukocyte activation antigen M6, a member of the Ig superfamily, is the species homologue of rat OX-47, mouse basigin, and chicken HT7 molecule, J. Immunol. 149 (1992) 847–854.
[5] H. Seulberger, C.M. Unger, W. Risau, HT7, Neurothelin, Basigin, gp42 and OX-47 – many names for one developmentally regulated immuno-globulin-like surface glycoprotein on blood–brain barrier endothelium, epithelial tissue barriers and neurons, Neurosci. Lett. 140 (1992) 93–97.
[6] W.C. Tsai, Y.C. Chao, W.H. Lee, A. Chen, L.F. Sheu, J.S. Jin, Increasing EMMPRIN and matriptase expression in hepatocellular carcinoma: tissue microarray analysis of immunohistochemical scores with clinicopathological parameters, Histopathology 49 (2006) 388–395.
[7] J.J. van den Oord, L. Paemen, G. Opdenakker, C. de Wolf-Peeters, Expression of gelatinase B and the extracellular matrix metalloproteinase inducer EMMPRIN in benign and malignant pigment cell lesions of the skin, Am. J. Pathol. 151 (1997) 665–670.
[8] M. Polette, C. Gilles, V. Marchand, M. Lorenzato, B. Toole, J.M. Tournier, S. Zucker, P. Birembaut, Tumor collagenase stimulatory factor (TCSF) expression and localization in human lung and breast cancers, J. Histochem. Cytochem. 45 (1997) 703–709.
[9] S. Caudroy, M. Polette, J.M. Tournier, H. Burlet, B. Toole, S. Zucker, P. Birembaut, Expression of the extracellular matrix metalloproteinase inducer (EMMPRIN) and the matrix metalloproteinase-2 in bronchopulmonary and breast lesions, J. Histochem. Cytochem. 47 (1999) 1575–1580.
[10] K. Muraoka, K. Nabeshima, T. Murayama, C. Biswas, M. Koono, Enhanced expression of a tumor-cell-derived collagenase-stimulatory factor in urothelial carcinoma: its usefulness as a tumor marker for bladder cancers, Int. J. Cancer 55 (1993) 19–26.
[11] S. Riethdorf, N. Reimers, V. Assmann, J.W. Kornfeld, L. Terracciano, G. Sauter, K. Pantel, High incidence of EMMPRIN expression in human tumors, Int. J. Cancer 119 (2006) 1800–1810.
[12] Y. Tang, P. Kesavan, M.T. Nakada, L. Yan, Tumor–stroma interaction: positive feedback regulation of extracellular matrix metalloproteinase inducer (EMMPRIN) expression and matrix metalloproteinase-dependent generation of soluble EMMPRIN, Mol. Cancer Res. 2 (2004) 73–80.
[13] J. Sun, M.E. Hemler, Regulation of MMP-1 and MMP-2 production through CD147/extracellular matrix metalloproteinase inducer interactions, Cancer Res. 61 (2001) 2276–2281.
[14] U. Albrecht, H. Seulberger, H. Schwarz, W. Risau, Correlation of blood–brain barrier function and HT7 protein distribution in chick brain circumventricular organs, Brain Res. 535 (1990) 49–61.
[15] C. Koch, G. Staffler, R. Huttinger, I. Hilgert, E. Prager, J. Cerny, P. Steinlein, O. Majdic, V. Horejsi, H. Stockinger, T cell activation-associated epitopes of CD147 in regulation of the T cell response, and their definition by antibody affinity and antigen density, Int. Immunol. 11 (1999) 777–786.
[16] T.C. Major, L. Liang, X. Lu, W. Rosebury, T.M. Bocan, Extracellular matrix metalloproteinase inducer (EMMPRIN) is induced upon monocyte differentiation and is expressed in human atheroma, Arterioscler. Thromb. Vasc. Biol. 22 (2002) 1200–1207.
[17] A.D. Marmorstein, S.C. Finnemann, V.L. Bonilha, E. Rodriguez-Boulan, Morphogenesis of the retinal pigment epithelium: toward understanding retinal degenerative diseases, Ann. NY Acad. Sci. 857 (1998) 1–12.
[18] R. DeCastro, Y. Zhang, H. Guo, H. Kataoka, M.K. Gordon, B. Toole, G. Biswas, Human keratinocytes express EMMPRIN, an extracellular matrix metalloproteinase inducer, J. Invest. Dermatol. 106 (1996) 1260–1265.
[19] L. Chen, M. Nakai, R.J. Belton Jr., R.A. Nowak, Expression of extracellular matrix metalloproteinase inducer and matrix metalloproteinases during mouse embryonic development, Reproduction 133 (2007) 405–414.
[20] E.E. Gabison, T. Hoang-Xuan, A. Mauviel, S. Menashi, EMMPRIN/CD147, an MMP modulator in cancer, development and tissue repair, Biochimie 87 (2005) 361–368.
[21] G. Pistol, C. Matache, A. Calugaru, C. Stavaru, S. Tanaseanu, R. Ionescu, S. Dumitrache, M. Stefanescu, Roles of CD147 on T lymphocytes activation and MMP-9 secretion in systemic lupus erythematosus, J. Cell Mol. Med. 11 (2007) 339–348.
[22] P. Zhu, J. Ding, J. Zhou, W.J. Dong, C.M. Fan, Z.N. Chen, Expression of CD147 on monocytes/macrophages in rheumatoid arthritis: its potential role in monocyte accumulation and matrix metalloproteinase production, Arthritis Res. Ther. 7 (2005) R1023–R1033.
[23] Y. Tang, M.T. Nakada, P. Kesavan, F. McCabe, H. Millar, P. Rafferty, P. Bugelski, L. Yan, Extracellular matrix metalloproteinase inducer stimulates tumor angiogenesis by elevating vascular endothelial cell growth factor and matrix metalloproteinases, Cancer Res. 65 (2005) 3193–3199.
[24] D. Millimaggi, M. Mari, S. D'Ascenzo, E. Carosa, E.A. Jannini, S. Zucker, G. Carta, A. Pavan, V. Dolo, Tumor vesicle-associated CD147 modulates the angiogenic capability of endothelial cells, Neoplasia 9 (2007) 349–357.
[25] M. Mutin, F. Dignat-George, J. Sampol, Immunologic phenotype of cultured endothelial cells: quantitative analysis of cell surface molecules, Tissue Antigens 50 (1997) 449–458.
[26] L. Bordenave, C. Baquey, R. Bareille, F. Lefebvre, C. Lauroua, V. Guerin, F. Rouais, N. More, C. Vergnes, J.M. Anderson, Endothelial cell compatibility testing of three different Pellethanes, J. Biomed. Mater. Res. 27 (1993) 1367–1381.
[27] H. Kataoka, R. DeCastro, S. Zucker, C. Biswas, Tumor cell-derived collagenase-stimulatory factor increases expression of interstitial collagenase, stromelysin, and 72-kDa gelatinase, Cancer Res. 53 (1993) 3154–3158.
[28] H.Y. Xu, A.R. Qian, P. Shang, J. Xu, L.M. Kong, H.J. Bian, Z.N. Chen, siRNA targeted against HAb18G/CD147 inhibits MMP-2 secretion, actin and FAK expression in hepatocellular carcinoma cell line via ERK1/2 pathway, Cancer Lett. 247 (2007) 336–344.
[29] J. Xu, H.Y. Xu, Q. Zhang, F. Song, J.L. Jiang, X.M. Yang, L. Mi, N. Wen, R. Tian, L. Wang, H. Yao, Q. Feng, Y. Zhang, J.L. Xing, P. Zhu, Z.N. Chen, HAb18G/CD147 functions in invasion and metastasis of hepatocellular carcinoma, Mol. Cancer Res. 5 (2007) 605–614.
[30] M. Maatta, T. Tervahartiala, K. Kaarniranta, Y. Tang, L. Yan, J. Tuukkanen, T. Sorsa, Immunolocalization of EMMPRIN (CD147) in the human eye and detection of soluble form of EMMPRIN in ocular fluids, Curr. Eye Res. 31 (2006) 917–924.
[31] T. Sameshima, K. Nabeshima, B.P. Toole, T. Inoue, K. Yokogami, S. Nakano, T. Ohi, S. Wakisaka, Correlation of emmprin expression in vascular endothelial cells with blood–brain-barrier function: a study using magnetic resonance imaging enhanced by Gd-DTPA and immunohistochemistry in brain tumors, Virchows. Arch. 442 (2003) 577–584.
[32] S. Zhou, H. Zhou, P.J. Walian, B.K. Jap, Regulation of gamma-secretase activity in Alzheimer's disease, Biochemistry 46 (2007) 2553–2563.
[33] T. Renno, A. Wilson, C. Dunkel, I. Coste, K. Maisnier-Patin, A. Benoit de Coignac, J.P. Aubry, R.K. Lees, J.Y. Bonnefoy, H.R. MacDonald, J.F. Gauchat, A role for CD147 in thymic development, J. Immunol. 168 (2002) 4946–4950.
[34] E.E. Gabison, S. Mourah, E. Steinfels, L. Yan, T. Hoang-Xuan, M.A. Watsky, B. De Wever, F. Calvo, A. Mauviel, S. Menashi, Differential expression of extracellular matrix metalloproteinase inducer (CD147) in normal and ulcerated corneas: role in epithelio–stromal interactions and matrix metalloproteinase induction, Am. J. Pathol. 166 (2005) 209–219.
[35] S. Menashi, M. Serova, L. Ma, S. Vignot, S. Mourah, F. Calvo, Regulation of extracellular matrix metalloproteinase inducer and matrix metalloproteinase expression by amphiregulin in transformed human breast epithelial cells, Cancer Res. 63 (2003) 7575–7580.
[36] H.C. Zheng, H. Takahashi, Y. Murai, Z.G. Cui, K. Nomoto, S. Miwa, K. Tsuneyama, Y. Takano, Upregulated EMMPRIN/CD147 might contribute to growth and angiogenesis of gastric carcinoma: a good marker for local invasion and prognosis, Br. J. Cancer 95 (2006) 1371–1378.
[37] Q. Zhang, J. Zhou, X.M. Ku, X.G. Chen, L. Zhang, J. Xu, G.S. Chen, Q. Li, F. Qian, R. Tian, N. Wen, Z.N. Chen, Expression of CD147 as a significantly unfavorable prognostic factor in hepatocellular carcinoma, Eur. J. Cancer Prev. 16 (2007) 196–202.
[38] X. Chen, J. Su, J. Chang, T. Kanekura, J. Li, Y.H. Kuang, S. Peng, F. Yang, H. Lu, J.L. Zhang, Inhibition of CD147 gene expression via RNA interference reduces tumor cell proliferation, activation, adhesion, and migration activity in the human jurkat T-lymphoma cell line, Cancer Invest. (2008) 1.
[39] S. Caudroy, M. Polette, B. Nawrocki-Raby, J. Cao, B.P. Toole, S. Zucker, P. Birembaut, EMMPRIN-mediated MMP regulation in tumor and endothelial cells, Clin. Exp. Metastasis 19 (2002) 697–702.
[40] N.A. Haseneen, G.G. Vaday, S. Zucker, H.D. Foda, Mechanical stretch induces MMP-2 release and activation in lung endothelium: role of EMMPRIN, Am. J. Physiol. Lung Cell Mol. Physiol. 284 (2003) L541–L547.

[41] N. Intasai, S. Mai, W. Kasinrerk, C. Tayapiwatana, Binding of multivalent CD147 phage induces apoptosis of U937 cells, Int. Immunol. 18 (2006) 1159–1169.
[42] M. Baba, M. Inoue, K. Itoh, Y. Nishizawa, Blocking CD147 induces cell death in cancer cells through impairment of glycolytic energy metabolism, Biochem. Biophys. Res. Commun. 374 (2008) 111–116.
[43] L. Wang, G. Wu, L. Yu, J. Yuan, F. Fang, Z. Zhai, F. Wang, H. Wang, Inhibition of CD147 expression reduces tumor cell invasion in human prostate cancer cell line via RNA interference, Cancer Biol. Ther. 5 (2006) 608–614.
[44] B.H. Jiang, L.Z. Liu, AKT signaling in regulating angiogenesis, Curr. Cancer Drug Targets 8 (2008) 19–26.
[45] K. Hamada, T. Sasaki, P.A. Koni, M. Natsui, H. Kishimoto, J. Sasaki, N. Yajima, Y. Horie, G. Hasegawa, M. Naito, J. Miyazaki, T. Suda, H. Itoh, K. Nakao, T.W. Mak, T. Nakano, A. Suzuki, The PTEN/PI3K pathway governs normal vascular development and tumor angiogenesis, Genes Dev. 19 (2005) 2054–2065.
[46] L. Hu, J. Hofmann, R.B. Jaffe, Phosphatidylinositol 3-kinase mediates angiogenesis and vascular permeability associated with ovarian carcinoma, Clin. Cancer Res. 11 (2005) 8208–8212.
[47] Y. Tang, M.T. Nakada, P. Rafferty, J. Laraio, F.L. McCabe, H. Millar, M. Cunningham, L.A. Snyder, P. Bugelski, L. Yan, Regulation of vascular endothelial growth factor expression by EMMPRIN via the PI3K-Akt signaling pathway, Mol. Cancer Res. 4 (2006) 371–377.
[48] D.Q. Zheng, A.S. Woodard, M. Fornaro, G. Tallini, L.R. Languino, Prostatic carcinoma cell migration via alpha(v)beta3 integrin is modulated by a focal adhesion kinase pathway, Cancer Res. 59 (1999) 1655–1664.
[49] Y. Cheng, X. Li, J. Kamholz, F.R. Burns, Organization of the mouse GP42/Basigin gene: a member of the Ig superfamily, Biochim. Biophys. Acta 1217 (1994) 307–311.