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Lymphatic-specific expression of dipeptidyl peptidase IV and its dual role in lymphatic endothelial function

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Abstract

Lymphatic vessels play an important role in the maintenance of tissue fluid homeostasis and in the transport of immune cells to lymph nodes, but they also serve as the major conduit for cancer metastasis to regional lymph nodes. However, the molecular mechanisms regulating these functions are poorly understood. Based on transcriptional profiling studies of cultured human dermal lymphatic (LEC) versus blood vascular endothelial cells (BEC), we found that dipeptidyl peptidase IV (DPPIV) mRNA and protein are much more strongly expressed by cultured lymphatic endothelium than by blood vascular endothelium that only expressed low levels of DPPIV in culture. The enzymatic cleavage activity of DPPIV was significantly higher in cultured LEC than in BEC. Differential immunofluorescence analyses of human organ tissue microarrays for DPPIV and several vascular lineage-specific markers revealed that DPPIV is also specifically expressed *in situ* by lymphatic vessels of the skin, esophagus, small intestine, breast and ovary. Moreover, siRNA-mediated DPPIV knockdown inhibited LEC adhesion to collagen type I and to fibronectin, and also reduced cell migration and formation of tube-like structures. These results identify DPPIV as a novel lymphatic marker and mediator of lymphatic endothelial cell functions.

Keywords

Lymphatic endothelial cells; lymphangiogenesis; DPPIV; cell migration

Introduction

The lymphatic vascular system is an open-ended network of endothelial cell-lined vessels that transport extravasated fluid, proteins, metabolites and cells from the interstitial space back to the circulatory system via the thoracic duct [1]. The lymphatic vessels also serve as the primary conduit for malignant tumor cell metastasis to regional lymph nodes, and induction of lymphangiogenesis by tumors actively promotes cancer metastasis [2–6]. There is increasing evidence that lymphatic vessels also actively participate in acute and chronic inflammation. The chronic inflammatory skin disease psoriasis is characterized by pronounced cutaneous lymphatic hyperplasia [7]. Kidney transplant rejection is frequently accompanied by lymphangiogenesis [8] and lymphangiogenesis has also been observed in

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experimental models of chronic airway inflammation [9]. However, the molecular mediators of lymphatic vessel function have remained poorly characterized.

During embryonic development, the transcription factor Prox1 plays a major role in the differentiation and sprouting of lymphatic progenitor cells from the cardinal veins [10]. Beginning at embryonic day (E) 9.5 of mouse development, Prox1 is specifically expressed by a subpopulation of endothelial cells that are located on one side of the anterior cardinal vein. These Prox1-positive LECs then bud from the veins to form the primary lymph sacs, which then proliferate and sprout into the periphery to form lymphatic capillaries and vessels - likely due to stimulation by vascular endothelial growth factor-C [1,11,12]. Budding and sprouting of LEC from the veins is arrested at ~E11.5–E12.0 in Prox1 null mice [12]. During later stages of development, several genes such as podoplanin [13], neuropilin-2 [14], FOX C2 [15] and angiopoietin-2 [16] are involved in regulating normal lymphatic vessel patterning and maturation.

Although some of these factors are also involved in lymphatic vessel activation under pathological conditions, the mechanisms controlling lymphatic vessel growth and function have remained poorly understood.

Dipeptidyl peptidase IV (DPPIV) is a membrane glycoprotein that cleaves conserved proline residues in proteotypically resistant components such as collagens, and that regulates the activities of a number of growth factors and neuropeptides [17–19]. DPPIV is involved in diverse biological processes, including cell differentiation, adhesion and apoptosis, functions that are also important for controlling neoplastic transformation [20–23]. In addition, DPPIV mediates binding to collagen [24,25], fibronectin and gelatin [26]. Despite its role in a number of cellular processes, the potential role of DPPIV for the growth and function of the lymphatic vascular system has remained unknown.

Based on transcriptional profiling studies that revealed an increased expression of DPPIV in cultured lymphatic endothelial cells (LEC) as compared to blood vascular endothelial cells (BEC), we aimed to characterize the vascular lineage-specific expression and function of DPPIV. We found – for the first time – that DPPIV expression is specifically expressed by lymphatic vessels but not by blood vessels in skin, as well as in a number of other organs including the small intestine, esophagus, ovary, peripheral nerve, breast and prostate glands. Studies in primary human LEC revealed that DPPIV is enzymatically active in these cells, but also promotes adhesion to fibronectin and collagen type I, as well as LEC migration and tube formation. These findings identify DPPIV as a novel lymphatic endothelium-specific marker, and they indicate that DPPIV plays a dual role in mediating lymphatic endothelial functions.

Materials and Methods

Cells

Primary human dermal lymphatic endothelial cells (LEC) and blood vascular endothelial cells (BEC) were isolated from neonatal human foreskins after routine circumcisions, as previously described [27]. The lineage-specific differentiation was confirmed by real-time RT-PCR for the lymphatic vascular markers Prox1, LYVE-1 and podoplanin, and for the blood vascular endothelial markers VEGFR-1 and VEGF-C, as well as by immunostains for CD31, LYVE-1 and Prox1 as described [27]. LEC were seeded onto fibronectin-coated culture dishes (10 µg/ml; BD Biosciences, Bedford, MA) and were cultured in endothelial cell basal medium (EBM; Cambrex Bio Science, Walkersville, MD) supplemented with 2 mM L-glutamine, 1% (v/v) antibiotic-antimycotic solution, 20% (v/v) fetal bovine serum

(FBS; all Invitrogen, Grand Island, N.Y.), 10 µg/ml hydrocortisone (Sigma, St. Louis, MO) and 2.5×10⁻² mg/ml N⁶,2'-O-dibutyryl-adenosine 3',5'-cyclic monophosphate (Sigma).

Quantitative RT-PCR and siRNA-transfections

The expression of DPPIV mRNA was quantified by real-time RT-PCR using the ABI 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). The probes and primers for DPPIV were pre-designed (Hs00175218_m1; Applied Biosystems). Each reaction was normalized for the expression of β-actin (forward primer 5'-TCACCGAGCGCGGCT-3', reverse primer 5'-TAATGTCACGCACGATTTC-3' and probe 5'-JOE-CAGCTTCACCACCACGGCCGAG-TAMRA-3') as an internal control. siRNA-transfection was performed using the Basic Nucleofector Kit for primary mammalian endothelial cells (Amaxa Biosystems, Cologne, Germany) according to the manufacturer's protocol. Predesigned siRNAs against human DPPIV (SI00030212, SI00030219, SI00030226; Qiagen, Hilden, Germany) and control siRNA (Silencer Negative Control #1 siRNA, Ambion, Cambridgeshire, UK) were used for the transfections.

Immunoblotting

For Western blot analysis of DPPIV protein expression, confluent cultures of LEC and BEC were homogenized in lysis buffer [28]. The protein concentrations were determined using the NanoOrange Protein Quantitation Kit (Molecular Probes, Eugene, OR). The lysates (100 µg of total protein) were then subjected to SDS-polyacrylamide gel electrophoresis using NuPAGE 10% BT gels, 1.0 mm, 12 well and NuPAGE MES SDS running buffer (20x) (Invitrogen). The proteins were transferred onto Trans-Blot Transfer Medium pure nitrocellulose membranes (BioRad, Hercules, CA) for immunoblot analysis. Blocking was performed with 5% non-fat dry milk in 0.1% Tween20 (Sigma) in PBS, followed by immunoblotting with a polyclonal goat anti-human DPPIV antibody (0.2 µg/ml, R&D Systems, Minneapolis, MN). Specific binding was detected by the ECL Plus Western Blotting Detection System (GE Healthcare, Buckinghamshire, UK). Equal loading was confirmed with an antibody against β-actin (Sigma).

Immunostains

Differential immunofluorescence stains using an antibody against DPPIV (R&D Systems; 1:100) together with antibodies against lymphatic-specific or blood vessel-specific markers were performed on 8-µm cryostat sections as described [7]. Stainings were performed using antibodies against the lymphatic-specific hyaluronan receptor LYVE-1 (Upstate/Millipore, Billerica, MA; 1:1000), the lymphatic-specific glycoprotein podoplanin [30] (D2-40; 1:200; Signet, Dedham, MA), the lymphatic-specific transcription factor Prox-1 (Covance, Princeton, NJ; 1:1000), the panendothelial marker CD31 (1:100, Dako Cytomation, Glostrup, Denmark) or the blood vessel-specific marker CD34 (BD Pharmingen, San Diego, CA; 1:100), and corresponding secondary antibodies labeled with AlexaFluor488 or AlexaFluor594 (Invitrogen/Molecular Probes). Nuclei were counterstained with 20 µg/ml of Hoechst bisbenzimidazole. Immunohistochemical stains were performed on tissue arrays of normal human tissues (MaxArray human normal tissue microarray slides, Zymed, San Francisco, CA) as described previously [29]. Briefly, the primary antibody against DPPIV (1:100) was applied, followed by incubation with conjugated anti-goat immunoglobulin using the 3-amino-9-ethylcabazole peroxidase kit (Vector Laboratories, Burlingame, CA). Additional immunofluorescence stains were performed using antibodies against DPPIV (1:100) and podoplanin (D2-40; 1:200), with corresponding secondary antibodies labeled with AlexaFluor594 and AlexaFluor488, respectively. Sections were examined using an AxioScope 2 mot plus (Carl Zeiss AG; Feldbach, Switzerland) and images were captured with a Zeiss AxioCam MRC.

DPPIV Enzyme Activity Assay

LEC or BEC were seeded into fibronectin-coated wells of 96-well plates in complete growth medium, at a cell density ranging from 20 to 20,000 cells/cm². After 24 hours, cells were washed twice with PBS, incubated with the DPPIV substrate Gly-Pro-aminoluciferin (DPPIV-Glo protease assay; Promega, Madison, WI), and gently mixed using a plate shaker at 500 rpm for 30 seconds. Plates were incubated for 2 hours in a buffer system optimized for DPPIV and luciferase activities. Luciferase activity was assessed by a LMAXII 384 luminometer (Bucher Biotec AG, Basel, Switzerland). For enzyme inhibition assays, 2,000 LEC were seeded into fibronectin-coated wells of 96-well plates in complete medium. After 20 hours, cells were washed twice with PBS and were treated with diprotin A (0.1 nM – 100 nM; International Peptides, Osaka, Japan) for 4 hours.

Cell migration, adhesion and tube formation assays

For endothelial cell migration assays, control or DPPIV siRNA-transfected LEC or BEC were grown to 100% confluency and serum starved overnight. The following day, a cell-free wound zone was created by scraping the monolayer with a sterile pipette tip. The cells were washed with PBS and the medium was changed to EBM containing either PBS or 3% FBS. The monolayers were incubated in 5% CO₂ at 37°C for 48 h. Representative images were taken at 5x magnification directly after wounding and after 48h, using an AxioCam MRm camera attached to an Axiovert 200M microscope (Carl Zeiss AG). Computer-assisted morphometric wound area analyses were performed using the IP-LAB software (Scanalytics, Fairfax, VA). For trans-well migration assays, 24-well FluoroBlock inserts of 8 µm pore size (BD Bioscience, Bedford, MA) were coated on the bottom side with 10 µg/ml fibronectin (BD Biosciences) for 1 h, followed by incubation with 100 µg/ml bovine serum albumin (BSA; Sigma) to block the remaining protein-binding sites. Cells (1×10⁶ cells/ml; 100 µl) were seeded in serum-free EBM medium containing 0.2% delipidized BSA into the upper chambers, and were incubated for 3 h at 37°C in the presence or absence of 3% FBS. Cells on the underside of inserts were stained with Calcein AM (Molecular Probes), and the fluorescence intensity was measured using a Spectra Max Gemini fluorescence reader (Bucher Biotec AG).

Cell adhesion assays were performed by coating 96-well plates with fibronectin (10 µg/ml) or type I collagen (50 µg/ml) for 30 min, followed by blocking with 100 µg/ml BSA. Control or DPPIV siRNA-transfected LEC or BEC (10⁵ cells in 200 µl of serum-free EBM) were seeded into each well and were incubated at 37°C for 45 min. Unattached cells were removed by three gentle washes with serum-free EBM containing 0.5% BSA; attached cells were stained with Calcein AM, fixed with 4% paraformaldehyde, and fluorescence was measured using a Spectra Max Gemini EM. Tube formation assays were performed as described [13]. Briefly, control or DPPIV siRNA transfected LEC were grown on collagen-coated 24 well plates until confluence. Then, 0.5 ml of neutralized isotonic bovine dermal collagen type I (2.6 mg/ml Vitrogen) with 3% FBS was added to the cells. After incubation at 37°C for 6 h, cells were fixed with 4% paraformaldehyde for 30 min at 4°C. Representative images were captured and the total length of tube-like structures per area was measured using the IP-LAB software as described [13]. All studies were repeated three times. Statistical analyses were performed using the unpaired Student's *t*-test.

Results

Enhanced expression of active DPPIV by LEC as compared to BVEC

To identify genes that are specifically expressed or up-regulated by LEC, as compared to blood vascular endothelial cells (BEC), we isolated and purified both LEC and BEC from human neonatal foreskins of three independent donors. The three LEC and BEC cell lines

were then subjected to transcriptional profiling by microarray analysis using Applied Biosystems Human Genome Survey 2.0 (Shin J. *et al.*, manuscript submitted). These studies revealed that DPPIV is expressed at higher levels by LEC than by BEC (23.1-fold average increase). The difference in DPPIV mRNA expression was confirmed by quantitative TaqMan real-time RT-PCR in three matched pairs of LEC and BEC that were obtained from the same donor, each with a more than 9-fold increase of DPPIV mRNA levels in LEC (Fig. 1A). After correction for the expression levels of beta-actin mRNA, the Ct values for DPPIV were between 7.54 and 8.71 in LEC and between 10.75 and 12.16 in BEC. Western blot analyses of cell lysates confirmed that the enhanced mRNA expression levels correlated with enhanced protein expression of DPPIV in LEC (Fig. 1B).

To further characterize the potential functional roles of DPPIV in LEC, we next investigated whether DPPIV produced by LEC is enzymatically active. Using a standard DPPIV activity assay for the cleavage of aminoluciferin, we found that the enzymatic activity of DPPIV was significantly higher in LEC than in BEC ($p < 0.001$), and that the activity increased with increasing cell numbers (Fig. 1C). The specificity of the enzymatic activity was confirmed by treatment of LEC with the DPPIV-specific inhibitor diprotin A, which resulted in a significant, dose-dependent repression of DPPIV cleavage activity (Fig. 1D).

Lymphatic vessels in normal skin specifically express DPPIV

To investigate whether DPPIV is also expressed by lymphatic vessels *in situ*, we next performed double immunofluorescence analyses of normal human skin for DPPIV and for the lymphatic markers LYVE-1, podoplanin and Prox1. LYVE-1-positive (Fig. 2B), podoplanin-positive (Fig. 2E) and Prox1-positive (Fig. 2N) lymphatic vessels also expressed DPPIV (Fig. 2 A–F, M–O). Immunofluorescent staining for the panendothelial marker CD31 revealed a complete overlap of DPPIV staining with the weakly stained CD31-lymphatic vessels (Fig. 2 G–I), whereas strongly stained CD31-positive blood vessels did not express DPPIV. In agreement with these findings, staining for the blood vascular-specific marker CD34 and for DPPIV was mutually exclusive (Fig. 2 J–L). Thus, DPPIV expression on blood vessels *in situ* was below the detection limit of the antibody used, and the low amount of *in vitro* expression by BEC might be due to the lack of environmental cues that are present *in situ*. Taken together, these findings confirm that DPPIV is specifically expressed by lymphatic vessels and not by blood vessels in human skin.

DPPIV is expressed by lymphatic vessels in several human organs

We next investigated whether DPPIV might also serve as a specific marker for lymphatic vessels in other human tissues, in addition to the skin. To this end, we analyzed human tissue microarrays containing sections of normal human organs. We found that lymphatic vessels in the small intestine, esophagus, ovary, breast, peripheral nerve tissue and prostate glands expressed DPPIV (Fig. 3 A–L). It is of interest that several glands, including the prostate (Fig. 3 K,L), salivary glands, and adrenal glands (data not shown) showed high expressions of DPPIV by glandular epithelium. Moreover, liver hepatocytes, proximal tubules of the kidney and bile ducts of the liver were also positive for DPPIV (data not shown). In all human tissues examined, DPPIV-positive lymphatic endothelium also expressed the lymphatic-specific marker podoplanin (Fig. 3 M,N), whereas blood vessels were DPPIV-negative.

SiRNA-mediated knockdown of DPPIV inhibits LEC adhesion and trans-well migration

We next investigated whether DPPIV inhibition might regulate LEC functions that are involved in lymphangiogenesis, including cell adhesion, migration and tube formation, using siRNA-mediated DPPIV knockdown. Using DPPIV-specific siRNA and Amaxa nucleofection, we achieved a >80% knockdown of DPPIV mRNA expression in LEC and

BEC, as determined by quantitative real-time RT-PCR (Fig. 4A and 6A). DPPIV siRNA knockdown inhibited the adhesion of LEC to both fibronectin and to collagen type I, as compared to control LEC ($P < 0.005$; Fig. 4B). In contrast, BEC adhesion to fibronectin was not inhibited by DPPIV siRNA knockdown, whereas BEC adhesion to collagen type I was moderately inhibited (Fig. 6B). Trans-well migration assays revealed that LEC transfected with DPPIV siRNA migrated significantly less efficiently towards a FBS gradient when compared to control LEC ($P < 0.005$; Fig. 4C). However, treatment with diprotin A (ranging from 0.01 nM to 10 nM) did not affect LEC adhesion and migration as compared to untreated controls (data not shown).

SiRNA-mediated knockdown of DPPIV delays *in vitro* wound closure and LEC tube-formation

We next tested whether DPPIV knockdown also inhibited LEC migration in a monolayer scratch wounding assay. Indeed, DPPIV siRNA knockdown significantly delayed wound closure of LEC as compared to control siRNA ($P < 0.0005$; Fig. 5A, B, C). In contrast, DPPIV siRNA knockdown did not inhibit wound closure by BEC (Fig. 6C). Knockdown of DPPIV in LEC also inhibited the formation of tube-like structures after overlay of confluent cultures with a collagen type I gel (Fig. 5D, E, F). In contrast, knockdown of DPPIV or diprotin A did not affect LEC proliferation (data not shown).

Discussion

In a search for novel pathways involved in lymphatic vessel growth and function, we have used transcriptional profiling of cultured human dermal BEC and LEC to identify enhanced expression of DPPIV in lymphatic endothelium *in vitro*. These results were confirmed by quantitative real-time RT-PCR and by Western blot analyses. We also found that DPPIV promotes LEC adhesion, migration and tube formation.

DPPIV has been implicated in several pathological conditions such as rheumatoid arthritis, Grave's disease and tumor progression [23,31–34]. Furthermore, recent reports indicated that DPPIV might also play a role in endothelial cells [35,36]. In this study, we found that DPPIV is specifically expressed by lymphatic vessels but not by blood vessels in the skin and in a number of additional organs, including the small intestine, esophagus, ovary, peripheral nerve, breast, and prostate glands. In contrast, DPPIV was not detected on lymphatic vessels in the lung, kidney, uterus, liver and stomach (data not shown).

DPPIV has several functions, including serine peptidase activity, binding to the extracellular matrix, and complexing adenosine deaminase [17,37]. Each of these distinct functions, presumably mediated by distinct domains, might contribute to its role in lymphatic function. Our results indicate that DPPIV, expressed by LEC, efficiently cleaved the DPPIV substrate Gly-Pro-aminoluciferin, demonstrating that DPPIV expressed in LEC is enzymatically active and functional. DPPIV has the ability to cleave bioactive peptides such as CXCL12, RANTES, MDC and I-TAC [37–39]. Therefore, DPPIV expressed by lymphatic vessels may contribute to the activation or deactivation of chemokines which control trafficking of monocytes, lymphocytes and dendritic cells into lymph nodes via lymphatic vessels. Whereas this enzymatic activity of DPPIV was efficiently inhibited by diprotin A, LEC proliferation and migration were not affected. However, we found that siRNA knockdown of DPPIV significantly inhibited LEC adhesion to fibronectin and collagen type I. These results indicate a dual function of DPPIV in lymphatic endothelium: Whereas the peptidase activity modulates the activity of proinflammatory chemokines and other mediators, DPPIV also mediates the interaction of lymphatic vessels with the extracellular matrix, an essential feature for the efficient drainage function of lymphatic vessels and the interstitial transport of macromolecules [1,40,41]. Moreover, siRNA-mediated DPPIV knockdown also inhibited

LEC migration and tube formation which are essential for developmental and pathological lymphangiogenesis. These results are in agreement with previous studies which indicated that migration of other cell types was mediated by the adhesive properties of DPPIV [42,43]. Therefore, specifically targeting the adhesive domain of DPPIV might provide a novel strategy for inhibiting pathological lymphangiogenesis. Future studies are needed to investigate whether DPPIV might also play a role in the mediation of tumor-induced lymphangiogenesis and lymphatic metastasis.

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Fig. 1. Enhanced expression of active DPPIV by LEC as compared to BEC

(A) Quantitative real-time RT-PCR confirmed that three independently established lines of primary LEC (filled bars) expressed high levels of DPPIV as compared to primary BEC (open bars). (B) Western blot analyses of cell lysates confirmed that LEC expressed much higher levels of DPPIV protein than BEC (top lane). Western blot analyses for β -actin was performed to demonstrate equal loading (bottom lane). (C) The DPPIV enzymatic activity was significantly higher in LEC (filled circles) than in BEC (open circles) and increased with increasing cell numbers. (D) Treatment of LEC with the DPPIV-specific inhibitor diprotin A, significantly and dose dependently repressed DPPIV cleavage activity (from 0.1 nM to 1 nM); treatment of BEC with diprotin A slightly repressed DPPIV cleavage activity (from 0.1 nM to 1 nM). *** $P < 0.0001$.

Fig. 2. Specific detection of DPPIV expression by lymphatic endothelium in human skin
 Double immunofluorescence analyses of normal human skin for DPPIV (A,D,M; green) and for the lymphatic specific markers (B) LYVE-1, (E) D2-40/podoplanin and (N) Prox1 revealed co-localization (C, F, O). Immunofluorescent staining for the panendothelial marker CD31 revealed a complete overlap of DPPIV staining with the weakly stained CD31-lymphatic vessels (G–I), whereas strongly stained CD31-positive blood vessels did not express DPPIV. Similarly, stainings for DPPIV and for the blood vascular-specific marker CD34 was mutually exclusive (J–L). Scale bars: 100 μ m.

Fig. 3. DPPIV is expressed by lymphatic vessels in several human organs

We found DPPIV-positive lymphatic vessels in the small intestine (A,B), esophagus (C,D), ovary (E,F), breast (G,H), peripheral nerve (I,J) and prostate gland (K,L). Strong expression of DPPIV was also found in several glands such as those in the small intestine (A,B) and the prostate (K,L). Boxed areas in panels A,C,E,G,I and K are shown at higher magnification on panels B,D,F,H,J and L, respectively. Immunofluorescence stains of ovarian tissue for DPPIV (M; red) and podoplanin (N; green) revealed specific localization of DPPIV in podoplanin-positive lymphatic vessels. Scale bars: 100 μm.

Figure 4. Knockdown of DPPIV inhibits LEC adhesion and trans-well migration *in vitro*
 (A) A greater than 80% knockdown of DPPIV mRNA expression was achieved at 24h and 72h after transfection of LEC with DPPIV siRNA. (B) DPPIV siRNA knockdown inhibited the adhesion of LEC (filled bars) to both fibronectin and to collagen type I, as compared to control LEC (open bars). (C) Trans-well migration assays revealed that LEC transfected with DPPIV siRNA (filled bars) migrated significantly less efficiently towards a FBS gradient when compared to control LEC (open bars). *** $P < 0.0005$.

Figure 5. Knockdown of DPPIV delays *in vitro* wound closure and inhibits LEC tube formation
 (A) DPPIV knockdown also inhibited LEC migration in a monolayer scratch wounding assay. Representative images demonstrate delayed wound closure by LEC transfected with DPPIV siRNA (C) when compared to control-treated LEC (B). (D) Knockdown of DPPIV in LEC (F) also inhibited the formation of tube-like structures after overlay of confluent cultures with a collagen type I gel when compared to control LEC (E). Scale bars: 100 μm. ***P<0.0005.

Figure 6. Effects of DPPIV knockdown on BEC adhesion and migration *in vitro*

(A) A greater than 85% knockdown of DPPIV mRNA expression was achieved at 36h, 48h and 72h after transfection of BEC with DPPIV siRNA. (B) DPPIV siRNA did not inhibit the adhesion of BEC (filled bars) to fibronectin, as compared to control LEC (open bars), whereas the adhesion to collagen type I was moderately inhibited. ** $P < 0.001$. (C) DPPIV knockdown did not inhibit BEC migration in a monolayer scratch wounding assay.