DPP4 INHIBITORS PROMOTE BIOLOGICAL FUNCTIONS OF HUMAN ENDOTHELIAL PROGENITOR CELLS BY TARGETING THE SDF-1/CXCR4 SIGNALING PATHWAY

Feng Liu[1], Guo-Dong Huang[2,*], Jia-Zhen Tang[1], Yu-Huan Peng[3]

[1] *Department of Endocrinology, The First Affiliated Hospital of Nanchang University*, Nanchang 330006, China

[2] *Department of Integrated Traditional Chinese and Western Medicine, The First Affiliated Hospital of Nanchang University,* Nanchang 330006, China

[3] *Department of Pharmacy, The First Affiliated Hospital of Nanchang University*, Nanchang 330006, China

*Corresponding author: huangguodong2014@163.com

Abstract: Dipeptidyl peptidase 4 (DPP4) inhibitors (oral hypoglycemic agents) have beneficial effects during the early stages of diabetes. In this study, we evaluated the role of DPP4 inhibitors on the biological functions of cultured human endothelial progenitor cells (EPCs). After treating EPCs with the DPP4 inhibitors sitagliptin and vildagliptin, we examined the mRNA expression of DPP4, vascular endothelial growth factor (VEGF), VEGF receptor 2 (VEGFR-2), endothelial nitric oxide synthase (eNOS), caspase-3, stromal cell-derived factor-1 (SDF-1), chemokine (C-X-C motif) receptor 4 (CXCR4) were measured by RT-PCR. The protein expression of SDF-1 and CXCR4 was determined by Western blot; cell proliferation was tested by the MTT method, and DPP4 activity was determined by a DPP4 assay. Our results revealed that DPP4 expression and activity were inhibited following the treatment with various doses of DPP4 inhibitors. Cell proliferation and the expression of VEGF, VEGFR-2 and eNOS were upregulated, while cell apoptosis was inhibited by DPP4 inhibitors in a dose-dependent manner. DPP4 inhibitors activated the SDF-1/CXCR4 signaling pathway, shown by the elevated expression of SDF-1/CXCR4. This further proved that after the SDF-1/CXCR4 signaling pathway was blocked by its inhibitor ADM3100, the effects of DPP4 inhibitors on the proliferation and apoptosis, and the expression of VEGF, VEGFR-2 and eNOS of EPCs were significantly reduced. These findings suggest that DPP4 inhibitors promote the biological functions of human EPCs by upregulating the SDF-1/CXCR4 signaling pathway.

Key words: DPP4 inhibitor; EPCs; biological function; SDF-1; CXCR4

INTRODUCTION

DPP4, a member of the prolyoligopeptidase family, is a transmembrane glycoprotein with serine exopeptidase activity that cleaves X-proline dipeptides from the N-terminus of polypeptides such as chemokines, neuropeptides, and vasoactive peptides (Matteucci and Giampietro, 2009). DPP4 is known to be present in many tissues including bone marrow, kidney, lung, liver, spleen, pancreas, intestines and venular end of blood vessels (Augustyns et al., 1999; Lambeir et al., 2003), and it is also expressed on the surface of several cell types, including epithelial cells, embryonic stem cells, hematopoietic stem cells, hematopoietic progeni-

tor cells and memory T cells (Christopherson et al., 2004; Guo et al., 2005). DPP4 activity is associated with the development of obesity (Lamers et al., 2011), diabetes mellitus (Drucker et al., 2007), renal disease (Sato et al., 2014), cardiovascular disease (Jose et al., 2011; Zhong et al., 2013) and inflammation (Zhong et al., 2013). Therefore, it is very important to examine potential agents that target DPP4 in order to treat these diseases.

Previous studies reported that DPP4 inhibition exhibited protective effects on ischemia-reperfusion injury of the heart, lung and kidney in rats (Glorie et al., 2012). DPP4 inhibition also attenuated filtration

barrier injury and oxidant stress in the obese Zucker rat (Nistala et al., 2014), and prevented obesity-induced renal injury in male mice (Nistala et al., 2014). Additionally, DPP4 inhibition combined with luminal nutrients was an effective therapy for the treatment of small intestinal ulcers in rats (Fujiwara et al., 2015). DPP4 inhibitors such as sitagliptin, vildagliptin, saxagliptin, alogliptin and linagliptin provide a comparatively novel approach in diabetes treatment (Abad Paniagua et al., 2014; Kumar et al., 2014; Tomkin, 2014). Furthermore, several previous studies have suggested that DPP4 inhibitors directly or indirectly preserve renal function (Hocher et al., 2012; Sato et al., 2014), support cardiovascular functioning (Jose et al., 2011; Zhong et al., 2013), and prevent brain mitochondrial dysfunction and cognitive dysfunction caused by a high-fat diet (Pipatpiboon et al., 2013). The use of DPP4 inhibitors has opened new possibilities for the prevention and treatment of human diseases.

Numerous findings have demonstrated that endothelial dysfunction is a systemic process that is the first step in the pathogenesis of atherosclerosis and atherosclerotic plaque progression (Hattori et al., 2006). Endothelial progenitor cells (EPCs) can be used to repair tissues after myocardial infarction and contribute to therapeutic angiogenesis (Kawamoto et al., 2001). Many EPCs agonists, such as granulocyte-colony stimulating factor (G-CSF), VEGF and statins can mobilize EPCs in bone marrow (Rafii and Lyden, 2003). A well accepted hypothesis is that the chemokine SDF-1 and its main receptor CXCR4 are expressed in progenitor cells and play a pivotal role in EPC mobilization and homing (Peled et al., 1999; Zhou et al., 2014). However, little is known of the role of DPP4 inhibition in the biological functions of EPCs. Furthermore, whether there is a link between the SDF-1/CXCR4 axis and DPP4 inhibitors in EPCs remains unclear. In the present study we used an *in vitro*-cultured human EPCs as a model to evaluate the effects of DPP4 inhibitors on the biological functions of human EPCs and to investigate the mechanism of DPP4 inhibitors.

MATERIALS AND METHODS

Cell culture

The human endothelial progenitor cells (EPCs), obtained from the China Center for Type Culture Collection (Wuhan, China), were cultured in growth medium EBM-2 (Lonza Walkersville, USA, basal medium with 8 factors and 5% fetal bovine serum). Cells were propagated in a humidified environment at 37°C with 5% CO_2 and 100% humidity.

DPP activity

After human EPCs were assigned to ten groups receiving the DPP4 inhibitors sitagliptin (Carbosynth Limited, Berkshire, UK) and vildagliptin (LGM Pharma, FL, USA) at doses of 0 (control), 0.01, 0.1, 1 or 10 μM for 48 h. DPP activity was determined by SensoLyte AMC DPP4 Assay Kit (AnaSpec, Inc., Fremont, CA) according to the manufacturer's instructions. Briefly, a cellular lysate containing 10 μg/50 μL of protein was mixed with 50 μL of DPP substrate AMC. The mixture was then measured using a fluorescence plate reader with excitation at 354 nm and emission at 442 nm (Fluoro Skan Ascent, Thermo Fisher Scientific K.K., Kanagawa, Japan) after a 30-min incubation at room temperature. Cellular DPP activity was expressed as relative fluorescence units (RFU).

MTT methods

The endogenous effects of DPP4 inhibitors on cell proliferation were evaluated by methyl thiazolyl tetrazolium (MTT) method. In brief, human EPCs were seeded in 96-well plates at a density of 1.0×10^5/mL and treated with 0, 0.01, 0.1, 1 and 10 μM sitagliptin or vildagliptin. At 48 h, cell proliferation was evaluated by adding 15 μL of 5 mg MTT/mL (Sigma, USA) solution to each well of one cell culture plate and incubating for 4 h. After the medium was removed, 150 μL of dimethyl sulfoxide was added to each well, and the plate was agitated for 10 min on a shaker to dissolve formazan. Absorbance at 490 nm was determined using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Real-time RT-PCR

Following treatment with 0, 0.01, 0.1, 1 and 10 μM of the DPP4 inhibitors sitagliptin or vildagliptin for 48 h, the mRNA expression of VEGF, VEGFR-2, eNOS, caspase-3, SDF-1 and CXCR4 in human EPCs was determined at the indicated times quantitative reverse transcription polymerase chain reaction (RT-PCR). Briefly, total cellular RNA was isolated from cells on 6-well plates using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA quality was assessed by agarose gel electrophoresis, and complementary DNA (cDNA) was synthesized with a random hexamer (TaKaRa, Osaka, Japan). The real-time RT-PCR analysis was carried out using QuantiTect SYBR Green RT-PCR Kit (Qiagen, Valencia, CA) under an ABI Prism 7500 Sequence Detector (Applied Biosystems, Foster City, CA, USA) following the manufacturers' instructions. The conditions were as follows: 1 cycle of denaturation at 95°C for 30 s, followed by 42 cycles at 95°C for 5 s, 58°C for 40 s, 1 cycle at 95°C for 15 s, and 60°C for 30 s. Specific primer sequences were synthesized in BIOSUNE Biological Technology Corp (Shanghai, China), and sequences of the primers are shown in Table 1. Expression of target genes was normalized to the expression of β-actin.

Table 1. Primers used in this study

Gene	Serial number	Primers
β-actin	X00351.1	Sense: 5'-GCCGATCCACACGGAGTACT-3' Anti-sense: 5'-CTGGCACCCAGCACAATG-3'
DPP4	NM_001935.3	Sense:5'-AAATGGGATTTGTGGACA-GCAAG-3' Anti-sense:5'-CCGATCCCAGGACCATTGAG-3'
Caspase-3	NM_032991.2	Sense:5'-TATTCTTAAAGTATTTTCGTTAC-3' Antisense: 5'-TCGTTATATAGACAACTCGATA-3'
CXCR4	AY242129.1	Sense: 5'-CCTCGCCTTCTTCCACTGTT-3' Anti-sense: 5'-CTGGGCAGAGCTTTTGAACTTG-3'
SDF-1	NM_199168.3	Sense: 5'-GTGTCACTGGCGACACGTAG-3' Antisense: 5'-TCCCATCCCACAGAGAGAAG-3'
eNOS	BC069465.1	Sense:5'-CACCAAACGTCGGGACCCGG-3' Antisense: 5'-TATGTCCTGAGTCCTACCCG-3'
VEGF	M32977.1	Sense: 5'-TACCGTCTTCCTCCTCGAT-3' Antisense: 5'-CACTGTTCGGCTCCGCCA-3'
VEGFR2	EU826563.1	Sense: 5'-CGGAGCCAGTAAATCGATCAAG-3' Antisense: 5'-CCTCGAATTCTTACGTAGGAACG-3'

Caspase-3 activity assay

The caspase-3 colorimetric activity assay was performed according to the manufacturer's instructions. In brief, the reaction buffer and the specific enzyme DEVD-pNA were added to each sample and incubated for 1-2 h at 37°C. The developed colorimetric reaction was measured at 405 nm in a 96-well microplate reader (Biorad Model) and values plotted as arbitrary units.

Western blot assay

Western blot analysis was performed to measure the expression of CXCR4 and SDF-1. Briefly, proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred electrophoretically to PVDF membranes. Then the membranes were blocked in 5% non-fat milk for 2 h at room temperature and incubated at 4°C overnight with antibodies SDF-1 and CXCR4 (1:1000 dilution; R&D Systems, Minneapolis, MN, USA). After overnight incubation, the membranes were washed and immunoblotted with HRP-conjugated anti-rabbit IgG antibody (diluted 1:1000; Amersham Biosciences, Piscataway, NJ, USA) at 37°C for 1 h. The membranes were then developed using enhanced chemiluminescence (ECL) (Amersham, Buckinghamshire, UK) and exposed to X-ray film. Band density was quantitated using Image J software and normalized to the β-actin levels.

RESULTS

Effects of DPP4 inhibitors on the expression and activity of DPP4 in human EPCs

We first examined the differences in DPP mRNA expression of cultured human EPCs treated with DPP4 inhibitors. Real-time PCR analysis revealed that there were no significant changes in DPP4 mRNA expression after treatment with 0.01 μM sitagliptin or vildagliptin (*P*>0.05). However, DPP4 mRNA expression was reduced significantly by higher doses of sitagliptin or vildagliptin (*P*<0.05), and the effects were dose-
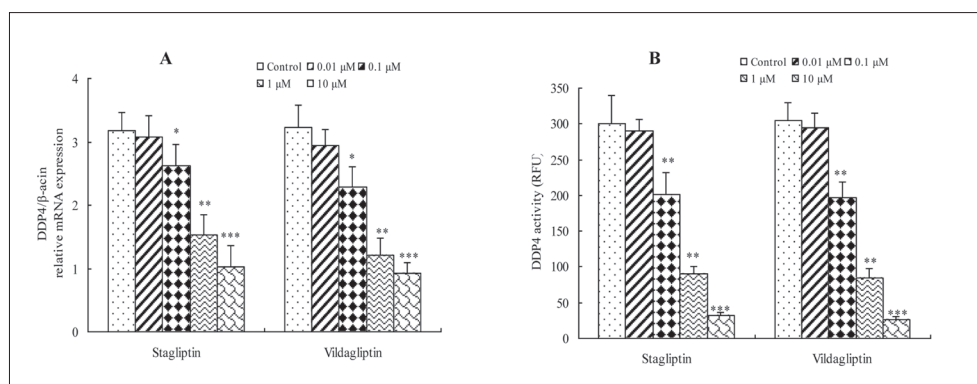
Fig. 1. The effects of DPP4 inhibitors on the expression and activity of DPP4 in human EPCs. Human EPCs were treated with 0, 0.01, 0.1, 1 and 10 μM of DPP4 inhibitors sitagliptin or vildagliptin for 48 h, after which mRNA expression (A) and activity (B) of DPP4 were measured. Data are expressed as mean±SD of three independent experiments performed in triplicate. $^{*}P<0.05$, $^{**}P<0.01$, or $P>0.05$ vs control.

dependent (Fig. 1A). Next, we measured the cellular DPP activity in human EPCs. The DPP activity assay revealed that, compared to the control, the distribution of DPP4 enzyme activity showed no difference in the 0.01-μM sitagliptin or vildagliptin group, while it was considerably decreased by sitagliptin or vildagliptin at concentrations of 0.1, 1 and 10 μM ($P<0.05$) (Fig. 1B). This suggested that DPP inhibitors could successfully inhibit the expression and enzyme activity of DPP4 in human EPCs, and that the effects of the higher dose of DPP4 inhibitors were more significant ($P<0.05$).

Effects of DPP4 inhibitors on the proliferation of human EPCs

To determine the influence of DPP4 inhibitors on the growth of human EPCs, cell proliferation was detected by MTT colorimetry. The data showed that, compared to the control without the stimulation of DPP4 inhibitors, DPP4 inhibitors sitagliptin or vildagliptin could promote the proliferation of human EPCs in a dose-dependent manner. Briefly, sitagliptin at concentrations of 0.01 and 0.1 μM or vildagliptin at a concentration of 0.01 μM increased the proliferation of human EPCs, however, the effects were not significant ($P>0.05$). Sitagliptin at concentrations of 1 and 10 μM or vildagliptin at concentrations of 0.1, 1 and 10 μM caused statistically significant cell growth stimulation ($P<0.05$). Furthermore, the effect of vildagliptin
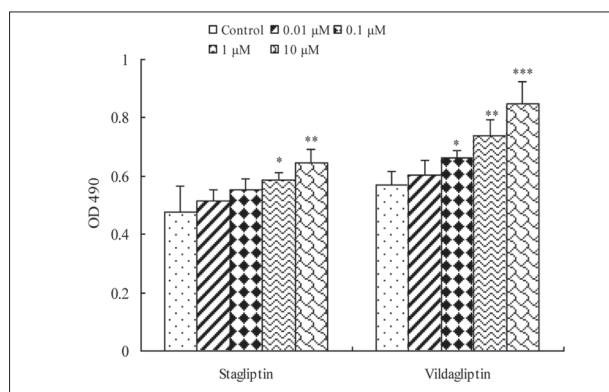

Fig. 2. The effect of DPP4 inhibitors on the proliferation of human EPCs. Human EPCs were treated with 0, 0.01, 0.1, 1 and 10 μM of DPP4 inhibitors sitagliptin or vildagliptin for 48 h, after which the proliferation of human EPCs was measured by the MTT method. Data are expressed as mean±SD of three independent experiments performed in triplicate. $^{*}P<0.05$, $^{**}P<0.01$, or $P>0.05$ vs control.

on cell proliferation was more significant than that of sitagliptin (Fig. 2).

Effects of DPP4 inhibitors on human EPC apoptosis

To determine the effect of DPP4 inhibitors on the apoptosis of human EPCs, mRNA expression and the activity of an apoptosis indicator, caspase-3, were examined in sitagliptin- or vildagliptin-treated human EPCs. There were no differences in caspase-3 mRNA expression (Fig. 3A) or activity (Fig. 3B) between the

control and the 0.01-µM sitagliptin- or vildagliptin-treated groups (*P*>0.05). Moreover, compared with the control group, mRNA expression and caspase-3 activity were significantly lower in the 0.1-, 1- and 10-µM sitagliptin- or vildagliptin-treated groups (*P*<0.05), and the effects were dose-dependent. These results indicated that DPP4 inhibitors could decrease the apoptosis of human EPCs.

Effects of DPP4 inhibitors on pro-angiogenic factors VEGF, VEGFR-2 and eNOS expression in human EPCs

The effects of DPP4 inhibitors on the pro-angiogenic factors VEGF, VEGFR2 and eNOS expression were investigated by RT-PCR in human EPCs. As shown in Fig. 4, this suggested that, compared to the control, 0.01 µM of sitagliptin or vildagliptin did not change the mRNA expression of VEGF, VEGFR2 and eNOS significantly (*P*>0.05), while sitagliptin (Fig. 4A) and vildagliptin (Fig. 4B) at concentrations of 0.1, 1 and 10 µM promoted mRNA expression of VEGF, VEGFR2 and eNOS in a dose-dependent manner (*P*<0.05), indicating that DPP4 inhibitors could enhance the angiogenesis of human EPCs.

Effects of DPP4 inhibitors on the activation of the SDF-1/CXCR4 signaling pathway in human EPCs

To understand the molecular mechanism involved in the regulatory functions of DPP4 inhibitors, the alterations in the expression of SDF-1 and CXCR4 in
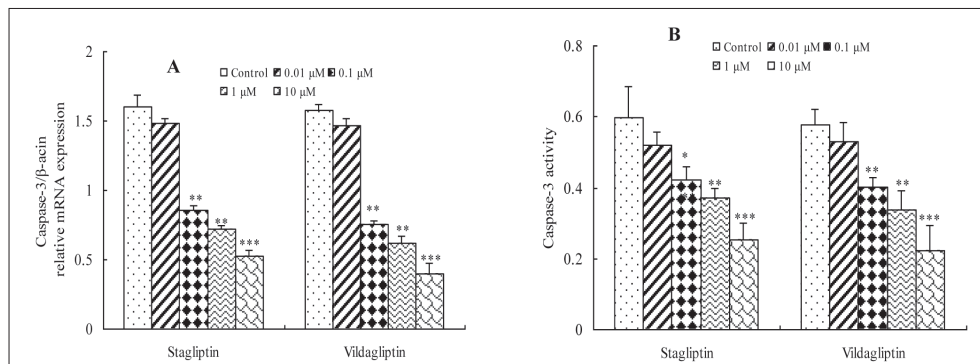

Fig. 3. The effect of DPP4 inhibitors on the apoptosis of human EPCs. Human EPCs were treated with 0, 0.01, 0.1, 1 and 10 µM of DPP4 inhibitors sitagliptin or vildagliptin for 48 h after which mRNA expression (A) and the activity (B) of caspase-3 in human EPCs were determined by RT-PCR and a caspase-3 kit. Data are expressed as the mean±SD of three independent experiments performed in triplicate. *P*<0.05, **P*<0.01, or *P*>0.05 vs control.
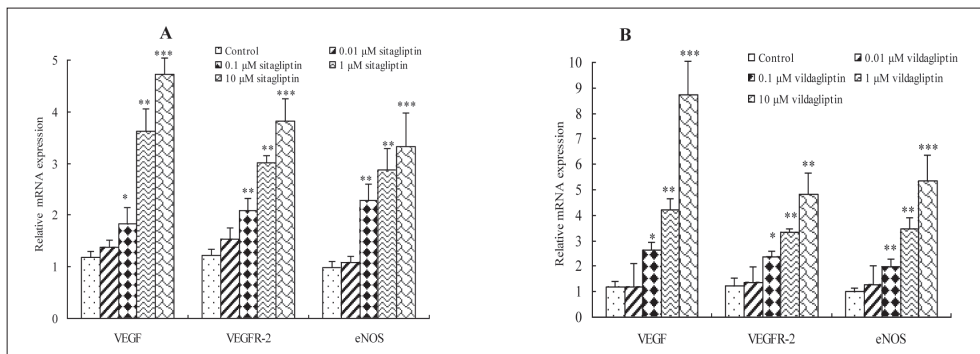

Fig. 4. Effect of DPP4 inhibitors on pro-angiogenic factors VEGF, VEGFR-2 and eNOS expression in human EPCs. After human EPCs were treated with 0, 0.01, 0.1, 1 and 10 µM of DPP4 inhibitors sitagliptin (A) or vildagliptin (B) for 48 h, mRNA expression of pro-angiogenic factors VEGF, VEGFR-2 and eNOS in human EPCs was determined by RT-PCR. Data are expressed as the mean±SD of three independent experiments performed in triplicate. *P*<0.05., **P*<0.01., or *P*>0.05 vs control.

human EPCs treated with 10 μM sitagliptin or vilda-
gliptin for 48 h were assessed using real-time RT-PCR
and Western blot analysis. We observed a marked
increase in SDF-1 and CXCR4 mRNA expression
(*P*<0.01) (Fig. 5A) and protein expression (*P*<0.05)
(Fig. 5B) following the treatment of sitagliptin or
vildagliptin. This indicated that DPP4 inhibitors could
activate the SDF-1/CXCR4 signaling pathway in hu-
man EPCs.

Role of the SDF-1/CXCR4 signaling pathway on the proliferation and apoptosis of DPP4 inhibitor-treated human EPCs

To evaluate the role of the SDF-1/CXCR4 signaling
pathway in DPP4 inhibitor-triggered biological func-
tions in human EPCs, the proliferation and apoptosis
of EPCs and the expression of pro-angiogenic factors
including VEGF, VEGFR2 and eNOS were investi-
gated following the blocking of the SDF-1/CXCR4 sig-
naling pathway activation by AMD3100. SDF-1 and
CXCR4 protein expression was significantly down-
regulated by AMD3100 (*P*<0.05), revealing the block-
ing efficiency of the AMD3100 inhibitor (Fig. 6A).

Furthermore, the DPP4 inhibitors sitagliptin and
vildagliptin promoted the proliferation and mRNA
expression of VEGF, VEGFR2 and eNOS while inhib-
iting the apoptosis of human EPCs (*P*<0.05). However,
there was no significant difference in cell proliferation
(Fig. 6B), apoptosis (Fig. 6C, D) of mRNA expres-
sion of VEGF, VEGFR2 and eNOS (Fig. 6E) (*P*>0.05)
between the control group and either the AMD3100
group or the group treated with the DPP4 inhibitor
and AMD3100. Taken together, these results strongly
suggest that the activation of the SDF-1/CXCR4 sig-
naling pathway was required for the DPP4 inhibitor-
triggered promotion of proliferation and decrease in
apoptosis in human EPCs.

DISCUSSION

DPP4, with a multiplicity of functions and targets,
can mediate the degradation of many chemokines and
neuropeptides, play a critical role in providing costim-
ulatory signals to T cells via adenosine deaminase and
regulate inflammatory responses in innate immune
cells such as monocytes and dendritic cells (Zhong et
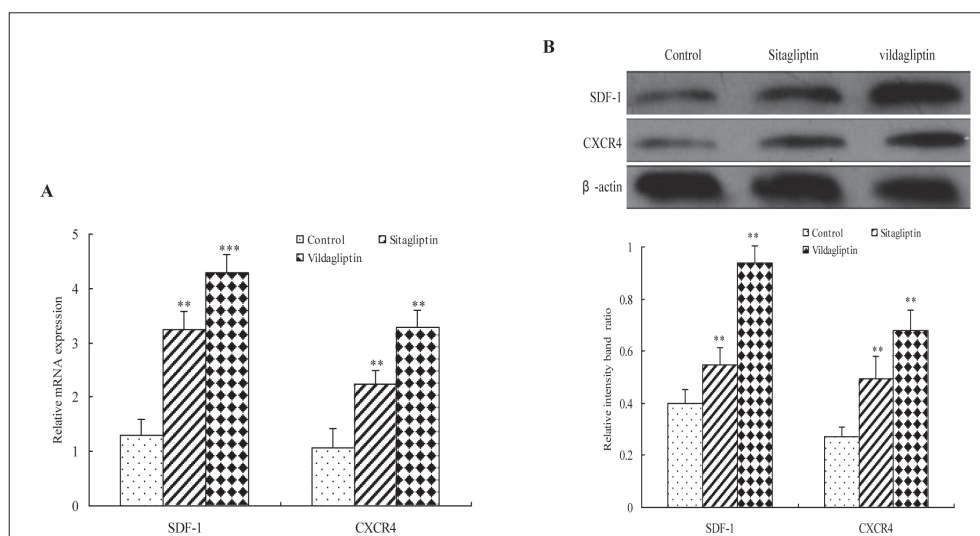al., 2013). Numerous *in vitro* and *in vivo* studies have

Fig. 5. Effect of DPP4 inhibitors on the activation of the SDF-1/CXCR4 signaling pathway in human EPCs.
Human EPCs were treated with 10 μM of DPP4 inhibitors sitagliptin or vildagliptin for 48 h, after which
the mRNA expression level of SDF-1/CXCR4 was detected by RT-PCR (A), and the protein expression
level of SDF-1/CXCR4 was determined by Western blot analysis (B). Data are expressed as the mean±SD
of three independent experiments performed in triplicate. *P<0.05., **P<0.01., or P>0.05 vs control.
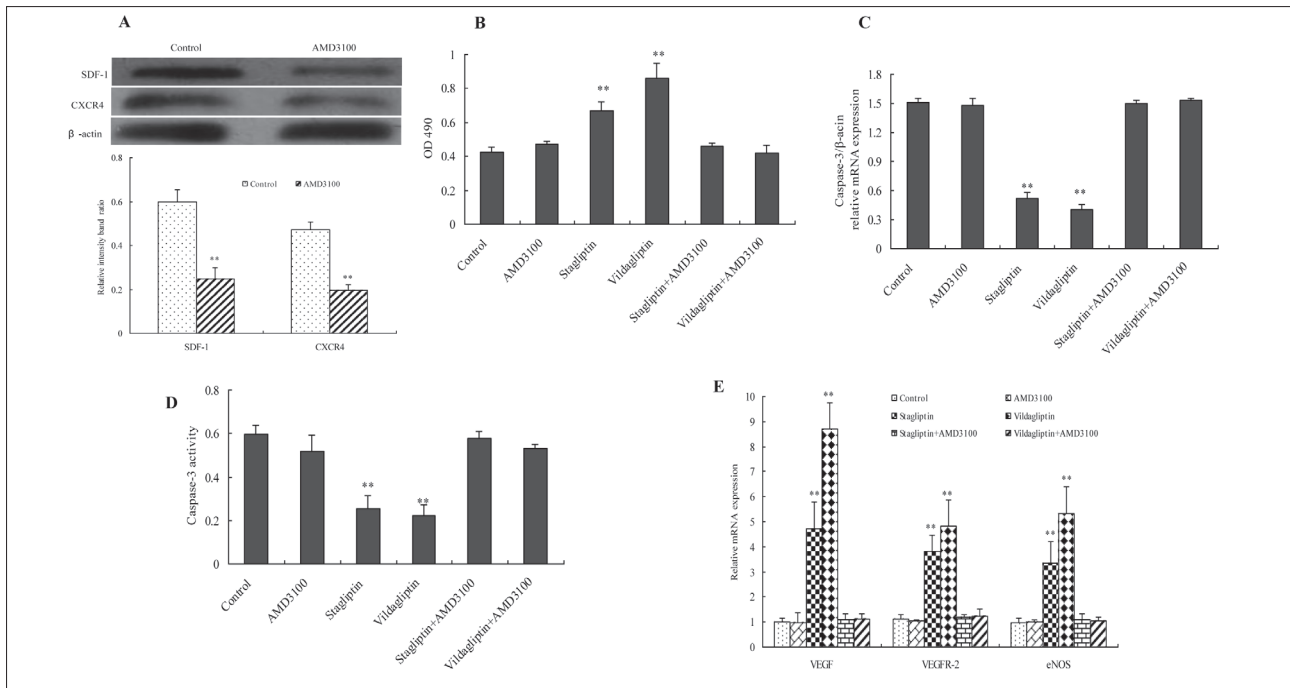
Fig. 6. Effect of DPP4 inhibitors on biological functions of human EPCs following the blocking of the SDF-1/CXCR4 signaling pathway. Cells were seeded in 6-well plates and then treated with 25 μM of AMD3100 for 48 h. Western blot was performed to determine the efficiency of the blocking of the SDF-1/CXCR4 signaling pathway (A). Cells attaining 85% confluency were treated with AMD3100 for 48 h, and 10 μM of DPP4 inhibitors sitagliptin or vildagliptin was added for 24 h, after which cell proliferation (B), mRNA expression (C) and the activity of Caspase-3 (D), mRNA expression of VEGF, VEGFR-2 and eNOS (E) were measured. Data are expressed as mean±SD of three independent experiments in triplicate. $^*P<0.05$, $^{**}P<0.01$, or $P>0.05$ vs control.

suggested that DPP4 activity is correlated with obesity, diabetes, heart failure and renal disease (Jose et al., 2011; Zhong et al., 2013; Sato et al., 2014). A recent *in vitro* study reported that DPP4 also possesses antithrombotic properties and may behave as an immobilized anticoagulant on endothelial cells (Hocher et al., 2012). DPP4 inhibitors such as sitagliptin, vildagliptin, saxagliptin, alogliptin and linagliptin have been used to cure diabetes, obesity, cardiovascular disease and kidney disease (Jose et al., 2011; Lamers et al., 2011; Zhong et al., 2013; Sato et al., 2014). In the present study, we evaluated the effects of DPP4 inhibitors on the biological functions of human EPCs and further investigated the mechanism of DPP4 inhibitors.

DPP4 expression and activity were found on the endothelium of intramyocardial blood vessels and endothelial cells (Hocher et al., 2012). Hyperglycemia is able to increase DPP4 activity and mRNA expression in a significant manner in microvascular endothelial cells (Pala et al., 2012). DPP4 inhibitors are currently used as glucose-lowering agents in type 2 diabetes, due to their effects on insulin and glucagon secretion. Several prospective clinical trials in humans have proven that the DPP4 inhibitor dapagliflozin alone or in combination with metformin, would be a cost-effective alternative in the treatment of type-2 diabetes mellitus (Abad Paniagua et al., 2014; Kumar et al., 2014). The expression level of DPP4 was significantly decreased by the addition of sitagliptin in human embryonic kidney 293 (HEK293) cells, human renal cancer cells (Sato et al., 2014), microvascular endothelial cells, and human umbilical vein endothelial cells (Pala et al., 2012). This study also indicated that the DPP4 inhibitors sitagliptin and vildagliptin could successfully inhibit the expression and enzyme activity of DPP4 in human EPCs.

The *in vivo* studies suggested that DPP4 inhibition resulted in the increased blood concentration of the incretin hormones glucagon-like peptide-1 (GLP-

1) and gastric inhibitory polypeptide (GIP), which caused an increase in glucose-dependent stimulation of insulin secretion, resulting in a lower blood glucose levels (Schmiedl et al., 2014). DPP4 inhibitors such as sitagliptin, vildagliptin, and saxagliptin worked by inducing a significant reduction in glycosylated hemoglobin levels (Doupis and Veves, 2008), slowing incretin metabolism, increasing endogenous GLP-1 concentrations and improving postprandial glycemic control in type 2 diabetes (Tomkin, 2014). Additionally, the DPP4 inhibitor vildagliptin preserved β-cell mass through an amelioration of endoplasmic reticulum stress in C/EBPB transgenic mice (Shimizu et al., 2012), and prevented neuronal insulin resistance by restoring insulin-induced long-term depression and neuronal IRS-1 phosphorylation, IR phosphorylation and Akt/PKB-Ser phosphorylation (Pipatpiboon et al., 2013). The DPP4 inhibitor linagliptin delayed the onset of diabetes and preserved β-cell mass in non-obese diabetic mice (Jelsing et al., 2012). In contrast, a study demonstrated that protein expressions of eNOS, CXCR4, SDF-1α and VEGF were remarkably higher in wild-type rats than in DPP4-deficient rats. Furthermore, vasorelaxation and nitric oxide production of the normal femoral artery were significantly reduced in DPP4-deficient than in wild-type Fischer rats, suggesting a positive role of DPP4 in maintaining vascular function and tissue perfusion in this experimental setting (Sun et al., 2013). The *in vitro* studies demonstrated that DPP4-inhibitor treatment facilitated an increase in hormones glucagon-like peptide-2 (GLP-2) receptor levels, intestinal growth and intestinal epithelial cell proliferation (Sueyoshi et al., 2014). Besides, DPP4-inhibition treatment enhanced engraftment of mouse bone marrow hematopoietic stem cells (Broxmeyer et al., 2013), and treatment with GLP-1 was able to increase the proliferation of the vasculoprotective EPCs as shown through an action on VEGF (Ku et al., 2011). Moreover, we found that the DPP4 inhibitors sitagliptin and vildagliptin promoted the proliferation of human EPCs and the expression of eNOS, CXCR4, SDF-1α, VEGFR-2 and VEGF in human EPCs, while they inhibited the apoptosis of this kind of cells. Why there is a difference between *in vivo* and *in vitro* studies still needs further investigation.

The SDF-1α/CXCR4 cascade is critical for the regulation of EPCs, and it might be an important therapeutic target for cardiovascular diseases, especially in myocardial infarction (Haider et al., 2008). As a natural substrate of DPP4, SDF-1 engineered to be resistant to DPP4 and matrix metalloproteinase-2 cleavage, and delivered by nanofibers, could improve blood flow in a model of peripheral artery disease (Segers et al., 2011). A study suggested that parathyroid hormone (PTH) inhibited DPP4, leading to an increased concentration of plasma SDF-1α and favoring the homing of bone-marrow-derived CXCR4+ EPCs to the sites of experimental myocardial infarction in mice (Huber et al., 2011). The cleavage of chemokines such as CXCL11, SDF-1 and eotaxin by DPP4 reduced the ability of these proteins to serve as chemoattractants to T cells and monocytes (Rizzo et al., 2009). In addition, SDF-1α and its receptor CXCR4 played a key role in the mobilization and migration of EPCs (Peled et al., 1999; Wang et al., 2006; Imanishi et al., 2008). After myocardial infarction, SDF-1α/CXCR4 interaction resulted in recruiting EPCs to the ischemic myocardium, increased EPC homing to the ischemic zone and participated in therapeutic angiogenesis (Haider et al., 2008; Frederick et al., 2010). Moreover, the experimental study of Herrera et al. (2001) demonstrated that the inhibition of DPP4 activity by angiotensin converting enzyme inhibitor (ACEI) increased circulating concentration and prolonged the biological half-life of SDF-1α, which, in turn, enhanced the circulating number of EPCs in an ischemic condition. Clinical observational studies have consistently shown that the inhibition of DPP4 activity by sitagliptin could increase circulating EPC levels in patients with type II diabetes mellitus (Fadini et al., 2010). The findings presented in the present study suggest that the SDF-1/CXCR4 signaling pathway was involved in the sitagliptin- or vildagliptin-induced proliferation and angiogenesis of human EPCs.

Together, our results suggest that the activation of the SDF-1/CXCR4 signaling pathway plays an important role in DPP4 inhibitor-induced promotion of human EPC growth and angiogenesis, and that it could serve as a potential therapeutic target in the treatment of cardiovascular diseases.

Conflict of interest disclosure: The authors declare that they have no conflict of interest.

REFERENCES

Abad Paniagua, E. J., Casado Escribano, P., Fernández Rodriguez, J. M., Morales Escobar, F. J., Betegón Nicolás, L., Sánchez-Covisa, J. and *M. Brosa* (2014). Cost-effectiveness analysis of dapagliflozin compared to DPP4 inhibitors and other oral antidiabetic drugs in the treatment of type-2 diabetes mellitus in Spain. *Aten. Primaria.* In press.

Augustyns, K., Bal, G., Thonus, G., Belyaev, A., Zhang, X. M., Bollaert, W., Lambeir, A. M., Durinx, C., Goossens, F. and *A. Haemers* (1999). The unique properties of dipeptidyl-peptidase IV (DPP IV/CD26) and the therapeutic potential of DPP IV inhibitors. *Current Med. Chem.* **6**, 311-327.

Broxmeyer, H. E. and *L. M. Pelus* (2014). Inhibition of DPP4/CD26 and dmPGE₂ treatment enhances engraftment of mouse bone marrow hematopoietic stem cells. *Blood. Cells. Mol. Dis.* **53**, 34-38.

Christopherson, K. W., Hangoc, G., Mantel, C. R., and *H. E. Broxmeyer* (2004). Modulation of hematopoietic stem cell homing and engraftment by CD26. *Science.* **305**, 1000-1003.

Doupis, J. and *A. Veves* (2008). DPP4 inhibitors: a new approach in diabetes treatment. *Adv. Ther.* **25**, 627-643.

Drucker, D. J. (2007). Dipeptidyl peptidase-4 inhibition and the treatment of type 2 diabetes: preclinical biology and mechanisms of action. *Diabetes Care.* **30**, 1335-1343.

Fadini, G. P., Boscaro, E., Albiero, M., Menegazzo, L., Frison, V., de Kreutzenberg, S., Agostini, C., Tiengo, A. and *A. Avogaro* (2010). The oral dipeptidyl peptidase-4 inhibitor sitagliptin increases circulating endothelial progenitor cells in patients with type 2 diabetes: possible role of stromal-derived factor-1alpha. *Diabetes Care.* **33**, 1607-1609.

Fujiwara, K., Inoue, T., Yorifuji, N., Iguchi, M., Sakanaka, T., Narabayashi, K., Kakimoto, K., Nouda, S., Okada, T., Ishida, K., Abe, Y., Masuda, D., Takeuchi, T., Fukunishi, S., Umegaki, E., Akiba, Y., Kaunitz, J. D. and *K. Higuchi* (2015). Combined treatment with dipeptidyl peptidase 4 (DPP4) inhibitor sitagliptin and elemental diets reduced indomethacin-induced intestinal injury in rats via the increase of mucosal glucagon-like peptide-2 concentration. *J. Clin. Biochem. Nutr.* **56**, 155-162.

Glorie, L. L., Verhulst, A., Matheeussen, V., Baerts, L., Magielse, J., Hermans, N., D'Haese, P. C., De Meester, I. and *A. De Beuf* (2012). DPP4 inhibition improves functional outcome after renal ischemia-reperfusion injury. *Am. J. Physiol. Renal. Physiol.* **303**, F681-688.

Guo, Y., Hangoc, G., Bian, H., Pelus, L. M. and *H. E. Broxmeyer* (2005). SDF-1/CXCL12 enhances survival and chemotaxis of murine embryonic stem cells and production of primitive and definitive hematopoietic progenitor cells. *Stem. Cells.* **23**, 1324-1332.

Haider, H. K., Jiang, S., Idris, N. M. and *M. Ashraf* (2008). IGF-1-overexpressing mesenchymal stem cells accelerate bone marrow stem cell mobilization via paracrine activation of SDF-1alpha/CXCR4 signaling to promote myocardial repair. *Circ. Res.* **103**, 1300-1308.

Herrera, C., Morimoto, C., Blanco, J., Mallol, J., Arenzana, F., Lluis, C. and *R. Franco* (2001). Comodulation of CXCR4 and CD26 in human lymphocytes. *J. Biol. Chem.* **276**, 19532-19539.

Hocher, B., Reichetzeder, C. and *M. L. Alter* (2012). Renal and cardiac effects of DPP4 inhibitors--from preclinical development to clinical research. *Kidney Blood Press. Res.* **36**, 65-84.

Huber, B. C., Brunner, S., Segeth, A., Nathan, P., Fischer, R., Zaruba, M. M., Vallaster, M., Theiss, H. D., David, R., Gerbitz, A. and *W. M. Franz* (2011). Parathyroid hormone is a DPP-IV inhibitor and increases SDF-1-driven homing of CXCR4+ stem cells into the ischaemic heart. *Cardiovasc. Res.* **90**, 529-537.

Imanishi, T., Tsujioka, H. and *T. Akasaka* (2008). Endothelial progenitor cells dysfunction and senescence: contribution to oxidative stress. *Curr. Cardiol. Rev.* **4**, 275-286.

Jelsing, J., Vrang, N., van Witteloostuijn, S. B., Mark, M. and *T. Klein* (2012). The DPP4 inhibitor linagliptin delays the onset of diabetes and preserves β-cell mass in non-obese diabetic mice. *J. Endocrinol.* **214**, 381-387.

Jose, T. and *S. E. Inzucchi* (2012). Cardiovascular effects of the DPP-4 inhibitors. *Diabetes Vas. Dis. Res.* **9**, 109-116.

Kawamoto, A., Gwon, H. C., Iwaguro, H., Yamaguchi, J. I., Uchida, S. Masuda, H., Silver, M., Ma, H., Kearney, M., Isner, J. M. and *T. Asahara* (2001). Therapeutic Potential of Ex Vivo Expanded Endothelial Progenitor Cells for Myocardial Ischemia. *Circulation.* **103**, 634-637.

Ku, H. C., Chen, W. P. and *M. J. Su* (2011) DPP4 deficiency preserves cardiac function via GLP-1 signaling in rats subjected to myocardial ischemia/reperfusion. *Naunyn Schmiedebergs Arch. Pharmacol.* **384**, 197-207.

Kumar, K. V. and *A. K. Gupta* (2014). Clinical audit of patients using DPP4 inhibitors in longstanding type 2 diabetes. *Diabetes Metab. Syndr.* In press.

Lambeir, A. M., Durinx, C., Scharpé, S. and *I. De Meester* (2003). Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV. *Crit. Rev. Clin. Lab. Sci.* **40**, 209-294.

Lamers, D., Famulla, S., Wronkowitz, N., Hartwig, S., Lehr, S., Ouwens, D. M., Eckardt, K., Kaufman, J. M., Ryden, M., Müller, S., Hanisch, F. G., Ruige, J., Arner, P., Sell, H. and *J. Eckel* (2011). Dipeptidyl peptidase 4 is a novel adipokine potentially linking obesity to the metabolic syndrome. *Diabetes.* **60**, 1917-1925.

Matteucci, E. and O. Giampietro (2009). Dipeptidyl peptidase-4 (CD26): knowing the function before inhibiting the enzyme. *Curr. Med. Chem*. **16**, 2943-2951.

Nistala, R., Habibi, J., Aroor, A., Sowers, J. R., Hayden, M. R., Meuth, A., Knight, W., Hancock, T., Klein, T., DeMarco, V. G. and A. Whaley-Connell (2014). DPP4 inhibition attenuates filtration barrier injury and oxidant stress in the Zucker obese rat. *Obesity* (Silver Spring). **22**, 2172-2179.

Nistala, R., Habibi, J., Lastra, G., Manrique, C., Aroor, A. R., Hayden, M. R., Garro, M., Meuth, A., Johnson, M., Whaley-Connell, A. and J. R. Sowers (2014). Prevention of obesity-induced renal injury in male mice by DPP4 inhibition. *Endocrinology*. **155**, 2266-2276.

Pala, L., Pezzatini, A., Dicembrini, I., Ciani, S., Gelmini, S., Vannelli, B. G., Cresci, B., Mannucci, E. and C. M. Rotella (2012). Different modulation of dipeptidyl peptidase-4 activity between microvascular and macrovascular human endothelial cells. *Acta Diabetologica*. **49**, S59-S63.

Peled, A., Grabovsky, V., Habler, L., Sandbank, J., Arenzana-Seisdedos, F., Petit, I., Ben-Hur, H., Lapidot, T. and R. Alon (1999). The chemokine SDF-1 stimulates integrin-mediated arrest of CD34(+) cells on vascular endothelium under shear flow. *J. Clin. Invest*. **104**, 1199-1211.

Peled, A., Petit, I., Kollet, O., Magid, M., Ponomaryov, T., Magid, M., Ponomaryov, T., Byk, T., Nagler, A., Ben-Hur, H., Many, A., Shultz, L., Lider, O., Alon, R., Zipori, D. and T. Lapidot (1999). Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science*. **283**, 845-848.

Pipatpiboon, N., Pintana, H., Pratchayasakul, W., Chattipakorn, N. and S. C. Chattipakorn (2013). DPP4-inhibitor improves neuronal insulin receptor function, brain mitochondrial function and cognitive function in rats with insulin resistance induced by high-fat diet consumption. *Eur. J. Neurosci*. **37**, 839-849.

Rafii, S. and D. Lyden (2003). Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. *Nat. Med*. **9**, 702-712.

Rizzo, M., Rizvi, A. A., Spinas, G. A., Rini, G. B. and K. Berneis (2009). Glucose lowering and anti-atherogenic effects of incretin-based therapies: GLP-1 analogues and DPP-4-inhibitors. *Expert Opinion Invest. Drugs*. **18**, 1495-1503.

Sato, Y., Kamada, T. and A. Yamauchi (2014). The role of dipeptidyl peptidase 4 (DPP4) in the preservation of renal function: DPP4 involvement in hemoglobin expression. *J. Endocrinol*. **223**, 133-142.

Schmiedl, A., Grützner, D., Hoffmann, T., von Hörsten, S. and M. Stephan (2014). DPP4 inhibitors increase differentially the expression of surfactant proteins in Fischer 344 rats. *Acta Physiol (Oxf)*. **212**, 248-261.

Segers, V. F. M., Revin, V., Wu, W., Qiu, H., Yan, Z., Lee, R. T. and A. Sandrasagra (2011). Protease-resistant stromal cell-derived factor-1 for the treatment of experimental peripheral artery disease. *Circulation*. **123**, 1306-1315.

Shimizu, S., Hosooka, T., Matsuda, T., Asahara, S., Koyanagi-Kimura, M., Kanno, A., Bartolome, A., Etoh, H., Fuchita, M., Teruyama, K., Takahashi, H., Inoue, H., Mieda, Y., Hashimoto, N., Seino, S. and Y. Kido (2012). DPP4 inhibitor vildagliptin preserves β-cell mass through amelioration of endoplasmic reticulum stress in C/EBPB transgenic mice. *J. Mol. Endocrinol*. **49**, 125-135.

Sueyoshi, R., Woods Ignatoski, K. M., Okawada, M., Hartmann, B., Holst, J. and D. H. Teitelbaum (2014). Stimulation of intestinal growth and function with DPP4 inhibition in a mouse short bowel syndrome model. *Am. J. Physiol. Gastrointest. Liver. Physiol*. **307**, G410-419.

Sun, C. K., Leu, S., Sheu, J. J., Tsai, T. H., Sung, H. C., Chen, Y. L., Chung, S. Y., Ko, S. F., Chang, H. W. and H. K. Yip (2013). Paradoxical impairment of angiogenesis, endothelial function and circulating number of endothelial progenitor cells in DPP4-deficient rat after critical limb ischemia. *Stem. Cell. Res. Ther*. **4**, 31.

Tomkin, G. H. (2014). Treatment of type 2 diabetes, lifestyle, GLP1 agonists and DPP4 inhibitors. *World. J. Diabetes*. **5**, 636-650.

Wang, C. H., Verma, S., Hsieh, I. C., Chen, Y. J., Kuo, L. T., Yang, N. I., Wang, S. Y., Wu, M. Y., Hsu, C. M., Cheng, C. W. and W. J. Cherng (2006). Enalapril increases ischemia-induced endothelial progenitor cell mobilization through manipulation of the CD26 system. *J. Mol. Cell. Cardiol*. **41**, 34-43.

Zhong, J., Rao, X., Deiuliis, J., Braunstein, Z., Narula, V., Hazey, J., Mikami, D., Needleman, B., Satoskar, A. R. and S. Rajagopalan (2013). A potential role for dendritic cell/macrophage-expressing DPP4 in obesity-induced visceral inflammation. *Diabetes*. **62**, 149-157.

Zhong, J., Rao, X. and S. Rajagopalan (2013). An emerging role of dipeptidyl peptidase 4 (DPP4) beyond glucose control: potential implications in cardiovascular disease. *Atherosclerosis*. **226**, 305-314.

Zhou, X., Porter, A. L., Robinson, D. K., Shim, M. S. and Y. Guo (2014). Nano-enabled drug delivery: a research profile. *Nanomedicine*. **10**, 889-896.