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Original Communication

Endothelium-specific up-regulation of heme oxygenase-1 by lentiviral transduction promotes release of positive regulators of adipocyte function, including Angiopoietin-1

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ABSTRACT

Crosstalk between perivascular adipose tissue and endothelial cells (EC) is thought to modulate vascular homeostasis, though the paracrine regulatory effects of dysfunctional endothelium on adipose tissue remain unclear. We explored the hypothesis that endothelial function affects adipocyte function. Our previous studies have shown that angiotensin II (AngII) causes endothelial dysfunction. Our preliminary results in mouse pre-adipocytes show that AngII increases lipogenesis. Consequently, human microvessel endothelial cells (HMEC-1, hereafter referred to as EC) were treated with AngII in the presence and absence of the heme oxygenase-1 (HO-1) inducer, Cobalt protoporphyrin (CoPP), with or without the HO activity inhibitor tin mesoporphyrin (SnMP). Ten percent conditioned media (CM) from EC was added to mesenchymal stem cell (MSC)-derived adipocytes. Adipocytes incubated with CM from AngII-treated EC showed a marked increase in adipogenesis, whereas adipocytes incubated with CM from AngII-treated EC concomitantly treated with CoPP attenuated adipogenesis. In contrast, CM from EC treated with AngII together with SnMP increased MSC-adipogenesis. We targeted vascular endothelial cells with a lentiviral (lenti) construct expressing human HO-1 under the control of an endothelium-specific promoter (VECAD-HO-1). MSCs exposed to CM from VECAD-HO-1-transduced EC exhibited a reduction in adipogenesis and lipid droplet size (10.2 ± 1 at 490 nm) as compared to MSCs exposed to CM from VECAD-GFP-treated EC (17.0 ± 2, $p < 0.05$). Furthermore, VECAD-HO-1-transduced EC expressed lower levels of soluble intercellular adhesion molecule 1 (sICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) when compared to control, suggesting that increased levels of EC HO-1 improved EC function. This effect was reversed by SnMP. Adipocytes treated with CM from VECAD-HO-1-transduced EC exposed to SnMP displayed increased adipogenesis and adipocyte hypertrophy (24.5 ± 1, $p < 0.01$). Finally, when CM from VECAD-HO-1-transduced cells was tested for paracrine growth factors, including vascular endothelial growth factor (VEGF) and Angiopoietin-1 (Angpt1), the latter proved most effective in ameliorating adipocyte dysfunction. Thus, increased HO-1 expression in EC attenuated adipogenesis and prevented adipocyte dysfunction.

KEYWORDS: endothelium, adipocytes, lentiviral transduction, Angiopoietin 1, obesity, heme oxygenase-1

INTRODUCTION

Oxidative stress is an important prequel to the development of endothelial dysfunction. Chronic

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redox imbalance is seen in the progression of many diseases including obesity, atherosclerosis, hypertension and diabetes mellitus [1-3]. The release of reactive oxygen species (ROS) by EC results in the release of inflammatory cytokines, which increase intercellular adhesion molecule 1 (ICAM-1) and VCAM expression. Given that up-regulation of adipogenesis requires extensive vascularization and that vascular dysfunction may affect adipogenesis, this process ultimately leads to adipocyte hypertrophy and dysfunction [4-9]. Adipocyte dysfunction can increase the release of angiotensin II (AngII), an important vasoactive hormone that activates cellular oxidases and potentiates redox imbalance [10, 11] and blockade of AngII has been shown to ameliorate adipocyte dysfunction [2, 12]. AngII also induces oxidative stress and contributes to endothelial dysfunction through the activation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system and generation of ROS in EC [13]. This mechanism may explain, in part, the process by which EC dysfunction modifies the adipocyte-mediated release of adipokines and thereby alters adipocyte phenotype [14, 15].

Obesity is a metabolic disorder and a risk factor commonly associated with endothelial dysfunction and the development of vascular disease in metabolic syndrome [16, 17]. Expansion of subcutaneous and visceral fat increases release of inflammatory cytokines, including monocyte chemoattractant protein 1 (MCP-1), interleukin 6 (IL-6), and tumor necrosis factor α (TNF-α), which contributes to vascular dysfunction in obesity [18]. Over time obesity-induced oxidative stress in EC results in chronic systemic inflammation [19] and increased adipogenesis, creating a vicious cycle in which adipocyte dysfunction causes EC dysfunction and *vice versa*. Moreover, while crosstalk between adipocytes and EC can promote dysfunction, they can also release factors which improve function.

Peroxisome proliferator-activated receptor-gamma (PPARγ) is a transcription factor considered to be the master regulator of adipogenesis [20-23]. Adipocyte-specific deletion of PPARγ leads to severe metabolic disturbances in mice and vascular studies have recognized PPARγ as an important suppressor of pro-inflammatory adhesion molecules in EC [24, 22, 8]. Furthermore, other growth factors released from EC, such as the vasculoprotective Angiopoietin 1 (Angpt1) [25-28], can ameliorate adipocyte dysfunction [29-32] and also inhibit MSC differentiation into adipocytes in favor of differentiation into chondrocytes and osteocytes [33].

Nephroblastoma overexpressed CCN3 (NOV/CCN3) is a recently discovered adipocytokine involved in insulin resistance and many other pathophysiological processes [34-36]. Elevated NOV levels are associated with increases in several inflammatory cytokines that might negatively impact insulin signaling, resulting in adipose tissue deposition and obesity and insulin resistance in patients with metabolic disorders [36]. In contrast, downregulation of NOV is associated with a reduction in adipose tissue deposition and inflammatory cytokines as well as enhanced insulin sensitivity in high fat diet (HFD)-fed mice, which is coupled with the up-regulation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) in adipose tissue [35]. We recently reported that an epoxyeicosatrienoic acid (EET) agonist not only induces HO-1 levels, but also downregulates NOV in adipose tissues of mice [37].

Induction of heme oxygenase 1 (HO-1) reduces adiposity [38-42] and oxidative stress, decreases excessive heme, and increases antioxidant enzymes such as superoxide dismutase and catalase [1, 39, 40, 43, 44]. HO consists of two forms, HO-1 (inducive) and HO-2 (constitutive) [45] and HO is responsible for the degradation of heme, resulting in the formation of carbon monoxide (CO), iron and biliverdin [1, 45, 46]. Increased expression of HO-1 enables cells to resist injury by heme and ROS. This protective effect of HO-1 is ascribed to several mechanisms: a reduction in cellular heme levels (a pro-oxidant), the induction of the iron-binding protein ferritin, the production of CO (anti-apoptotic), and the biliverdin/bilirubin (antioxidant) [1, 47-50]. Thus induction of HO-1 is central to antioxidant defense. However, high levels of glucose suppress HO-1 expression and HO activity in spite of ROS elevation in obesity [51, 52].

Previous studies of HO-1 induction reported a reduction in adipocyte hypertrophy, with a concomitant increase in adiponectin [39, 42, 53, 54] and small, healthy, insulin-sensitive adipocytes [55-57], while adipocyte-specific inhibition of

HO-1 in female mice causes metabolic abnormalities, including adipocyte hypertrophy, decreased mitochondrial biogenesis and function as well as reduced thermogenesis [37]. Therefore, HO-1 may play a role in preserving both leptin sensitivity and the action of other cytokines on the brain in regulating food intake and energy expenditure [58].

We induced HO-1 by transfecting EC with endothelium-specific lentivirus VECAD-HO-1 in order to examine the paracrine effect of vascular dysfunction on MSCs. The paracrine effect of endothelial oxidative stress on perivascular adipocytes is an important factor in the growth and differentiation of MSCs and pre-adipocytes [5]. Given that adipose tissue is highly vascularised by an extensive capillary network [5], we proposed that overexpression of endothelium-specific HO-1 by gene transfer attenuates O_2^- and AngII-mediated oxidative stress [11], and thereby attenuates AngII-induced vascular injury and dysfunction [52, 59-62]. The aim of our study was to examine crosstalk between EC and adipocytes in the regulation of adipogenesis. We show that increased HO-1 expression, both pharmacologically and by gene transfer, prevents adipocyte dysfunction by inhibiting adipogenesis. We moreover show that Angpt1, released by EC, inhibits adipocyte maturation and reduces expression of adipocyte and pro-inflammatory markers, including NOV.

MATERIALS AND METHODS

Cell culture and treatments

Human microvascular endothelial cells (EC) were purchased from ATCC (Manassas, VA) and maintained at 37 °C and 5% CO_2. EC were grown in endothelial cell growth medium (Lonza, Walkersville, MD) containing 1% penicillin/streptomycin and microvascular endothelial cell growth supplement until 80% confluent and treated with AngII in the presence and absence of the HO-1 inducer CoPP with or without the addition of the HO activity inhibitor SnMP.

For HO-1 overexpression, ECs were infected with 2 µl of lentiviral particles (10^9 transducing units (TU)/ml) (Lentigen, Baltimore, MD) carrying the HO-1 construct with GFP under the control of the vascular endothelium-specific promoter VECAD (VECAD-HO-1). Cells were transduced with the GFP construct alone (VECAD-GFP) as control and as previously described [63, 64]. GFP expression was confirmed using a confocal laser scanning (Fluoview FV300, Olympus, Center Valley, PA) microscope. The conditioned medium (CM) was collected and stored at -80 °C until analyzed.

Human bone marrow-derived mesenchymal stem cells (MSCs) purchased from AllCells (Emeryville, CA) and 3T3-L1 mouse pre-adipocytes (ATCC) were maintained at 37 °C and 5% CO_2 in α-MEM (Fisher Scientific, Waltham, MA) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. To induce adipogenesis MSCs were grown until 80% confluent in T-75 flasks and the medium was replaced with adipogenic medium (Dulbecco's Modified Eagle Medium (DMEM)-high glucose) (Fisher Scientific), 10% (v/v) FBS, 10 µg/ml insulin, 0.5 mM dexamethasone (Sigma Aldrich, St. Louis, MO) and 0.1 mM indomethacin (Sigma Aldrich). In some experiments, MSC-derived adipocytes were cultured in adipogenic differentiation media supplemented with 10% CM from the EC. Medium was changed every 2 days for 14 days. The CM from MSC-derived adipocytes was collected and stored at -80 °C until analyzed.

To induce adipogenesis 3T3-L1 cells were grown until fully confluent and the medium was replaced with adipogenic medium and cultured for another 5 days before subjected to gene expression analysis.

Oil Red O staining and lipid droplet size

MSC-derived adipocytes were fixed in 10% formaldehyde, incubated in Oil Red O solution (0.21% Oil Red O in 60% isopropanol) for 10 min, and then rinsed with 60% isopropanol. The Oil Red O was then eluted by incubation in 100% isopropanol for 10 min where after optical density (OD) was measured at 490 nm.

Western blot and real-time PCR analysis

For western blot analysis pelleted cells were lysed with lysis buffer supplemented with protease and phosphatase inhibitors (cOmplete™ Mini and PhosSTOP™, Roche Diagnostics, Indianapolis, IA). Immunoblotting for HO-1, ICAM-1, VCAM-1, CCAAT/enhancer-binding protein alpha (C/EBPα) and peroxisome proliferator-activated receptor gamma (PPAR-γ) and β-Actin was performed on cell and mouse adipose tissue lysates as previously

described [39, 40, 42]. Total RNA was extracted from cells using SpinSmart™ Total RNA purification kit (Denville Scientific Inc., Holliston, MA), as indicated by the manufacturer and RNA concentration was determined with a Biotek™ plate reader and the Take3™ plate (Biotek, Winooski, VT). cDNA was synthesized from total RNA (Fisher Scientific) using a High-Capacity cDNA Reverse Transcription Kit (Fisher Scientific) and real-time polymerase chain reaction (PCR) was performed using TaqMan® Fast Universal Master Mix (2x), on a 7500 HT Fast Real-Time PCR System (Fisher Scientific). Specific TaqMan® Gene Expression Assays probes for mouse NOV and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used as described previously [39, 40, 42].

Cytokine array

Protein levels of MCP-1, TNF-α, Angpt1, VEGF, and Endothelial Growth Factor (EGF) were determined in conditioned medium using enzyme-linked immunosorbent assays (Assay Gate, Ijamsville, MD) as previously described [65].

Statistical analyses

Data values are expressed as means ± S.E.M. Student's t-test was used for pairwise comparison and one-way analysis of variance (ANOVA) with Bonferroni's post-test for comparison was used to calculate the significance of mean value differences. The null hypothesis was rejected at $p < 0.05$.

RESULTS

Effect of AngII on adipogenesis

Figure 1 illustrates the effect of AngII on MSC-derived adipocytes. MSCs treated with AngII displayed a marked ($p < 0.05$) increase in adipogenesis (Figure 1A and B). In contrast, CoPP caused a significant ($p < 0.05$) decrease in adipogenesis when compared to AngII alone. SnMP prevented the decrease in adipogenesis seen with

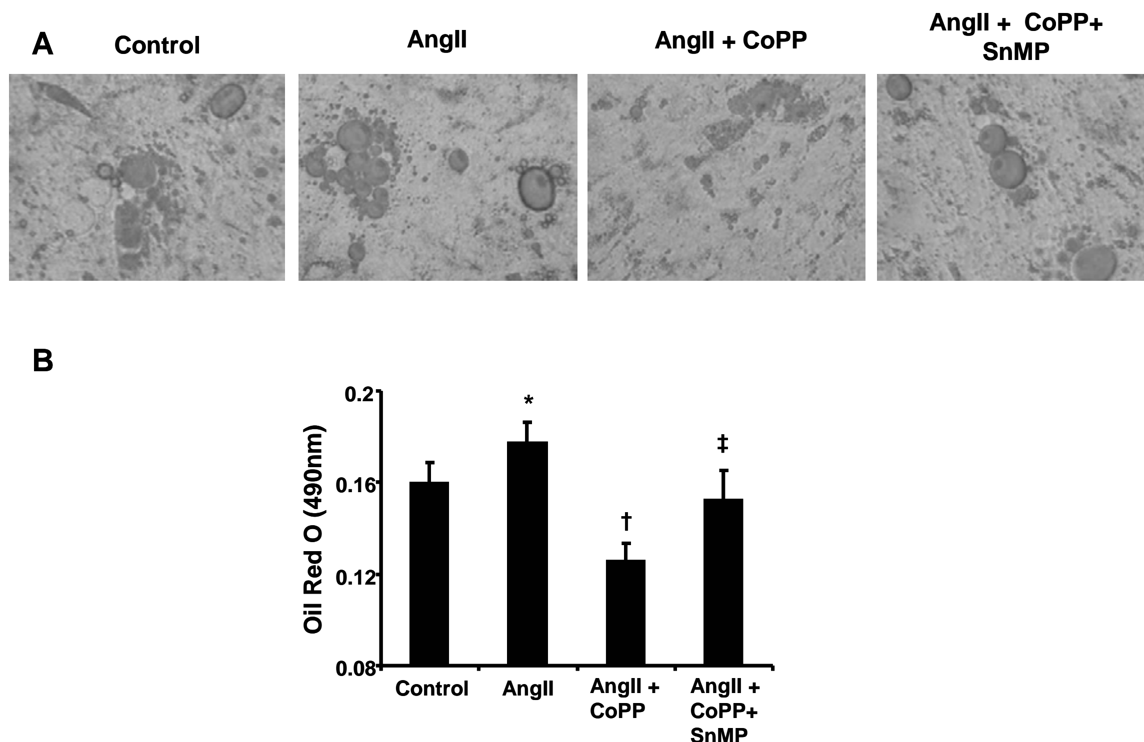

Figure 1. (A) Representative photographs of adipogenesis in MSC-derived adipocytes treated with CM from EC administered Ang-II, CoPP, and CoPP + SnMP. (B) Graph depicting the adipogenesis as measured by absorbance at 490 nm of Oil Red O incorporation into lipid droplets, $^{*}p < 0.05$ vs control, $^{\dagger}p < 0.05$ vs AngII, $^{\ddagger}p < 0.05$ vs AngII + CoPP.

CoPP alone and the levels of adipogenesis were similar to control (Figure 1A and B).

Effect of AngII on endothelial cell dysfunction

As seen in Figure 2, EC treatment with AngII did not change basal levels of HO-1 as compared to the control, whereas cells treated with AngII and CoPP resulted in an increase in HO-1 levels. HO-1 levels in cells treated concurrently with AngII, CoPP and SnMP were similar to those in cells treated with AngII and CoPP (Figure 2A and B). Treatment of EC with AngII increased the level of ICAM-1 as compared to control ($p < 0.05$) an effect that was prevented in cells concomitantly treated with CoPP (Figure 2A and C). The CoPP-mediated effect on ICAM-1 levels was effectively prevented in cells that were concomitantly treated with the HO-activity inhibitor, SnMP (Figure 2A and C).

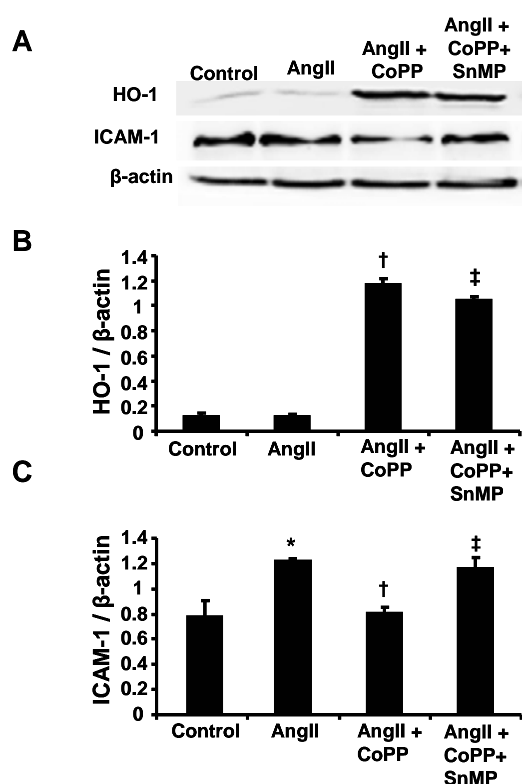

Figure 2. (A) Representative Western blots and densitometric analysis of (B) HO-1, and (C) ICAM-1 expression in conditioned medium from HMEC-1 treated with Ang-II, with or without CoPP or CoPP + SnMP, *$p < 0.05$ vs control, †$p < 0.05$ vs AngII, ‡$p < 0.05$ vs AngII + CoPP.

The viability of the lentivirus and its effect

In order to identify the relationship between EC function and adipocyte health, EC cells were transduced with either control (VECAD-LV-GFP) or HO-1-expressing (VECAD-HO-1-GFP) lentivirus. Fluorescence imaging (Figure 3A) demonstrates that the transduction was successful. As seen in Figure 3B and C, lenti-VECAD-HO-1 transduction increased protein expression of HO-1 and decreased expression of both ICAM-1 and VCAM-1 as compared to control. Addition of SnMP elevated ($p < 0.05$) ICAM-1 and VCAM-1 as compared to control (Figure 3B and C).

Analysis of biomarkers from EC-derived CM

We examined the levels of the inflammatory cytokine TNF-α, adhesion molecule sICAM-1, and the growth factors Angpt1 and VEGF. As seen in Figure 4C and D, CM from cells treated with VECAD-HO-1 resulted in an increase in Angpt1 and VEGF and a decrease in TNF-α and sICAM-1 (Figure 4A and B) when compared to the control. In contrast, CM from cells treated with VECAD-HO-1 and SnMP resulted in a decrease in Angpt1 and VEGF levels and an increase in TNF-α and sICAM-1 levels compared to control. EGF levels were unaffected by HO-1 up-regulation *via* gene transfer and SnMP (results not shown).

Effect of CM from EC on adipogenesis in MSCs

Figure 5A illustrates that adipogenesis is reduced in MSCs exposed to CM from VECAD-HO-1 EC, but that the reduction was not only reversed but increased to levels above those in the vehicle by treatment with SnMP. Figure 5B and C show the expression levels of HO-1 and the downstream adipocyte differentiation markers PPAR-γ and C/EBP-α in MSC-derived adipocytes. HO-1 expression in adipocytes was elevated with VECAD-HO-1 CM in the presence and absence of SnMP. Conversely, the expression of PPAR-γ and C/EBP-α was reduced by VECAD-HO-1 CM and increased by VECAD-HO-1 + SnMP CM.

Analysis of biomarkers from CM of adipocytes

We measured the ability of adipocytes pre-treated with CM from EC to release paracrine factors. In Figure 6, CM from ECs transduced with the

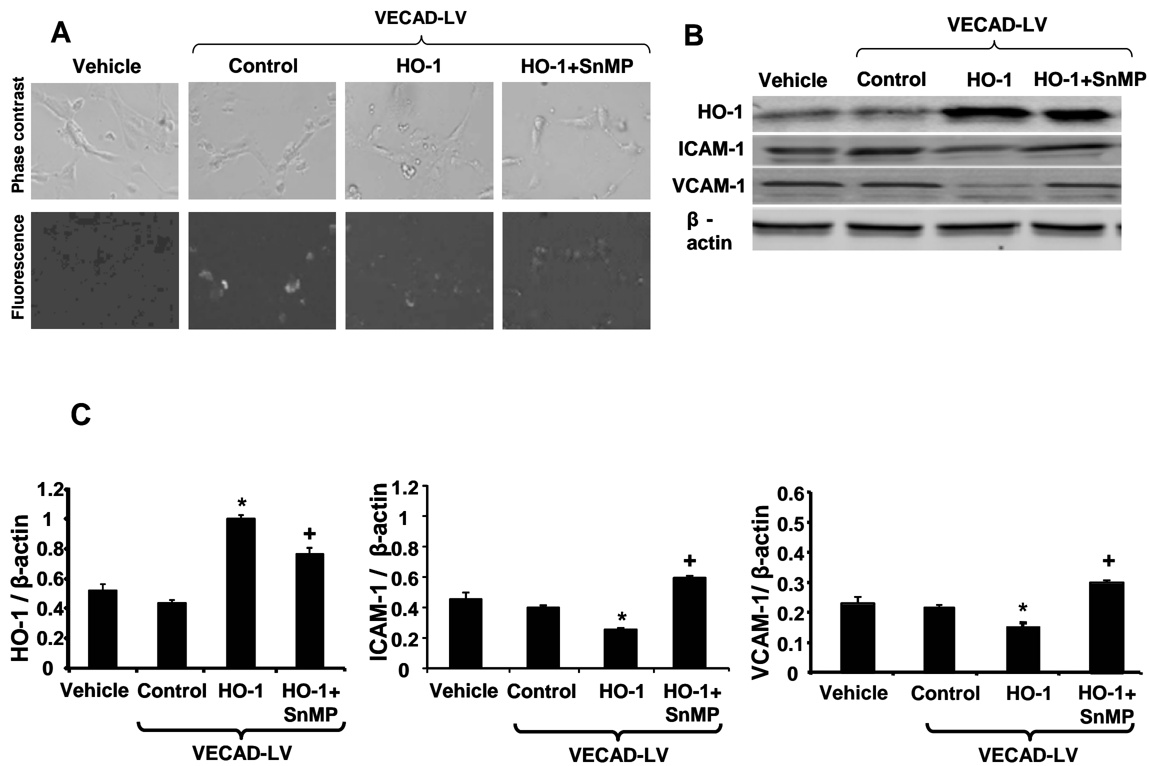

Figure 3. (A) Phase contrast and fluorescence imaging demonstrating the successful transduction of the VECAD lentivirus. (B) Representative Western blots and (C) densitometric analysis of HO-1, ICAM-1, and VCAM-1 expression following treatment of HMEC-1 with VECAD-GFP, VECAD-HO-1, and VECAD-HO-1 + SnMP, *$p < 0.05$ vs GFP (control), $^{+}p < 0.05$ vs HO-1.

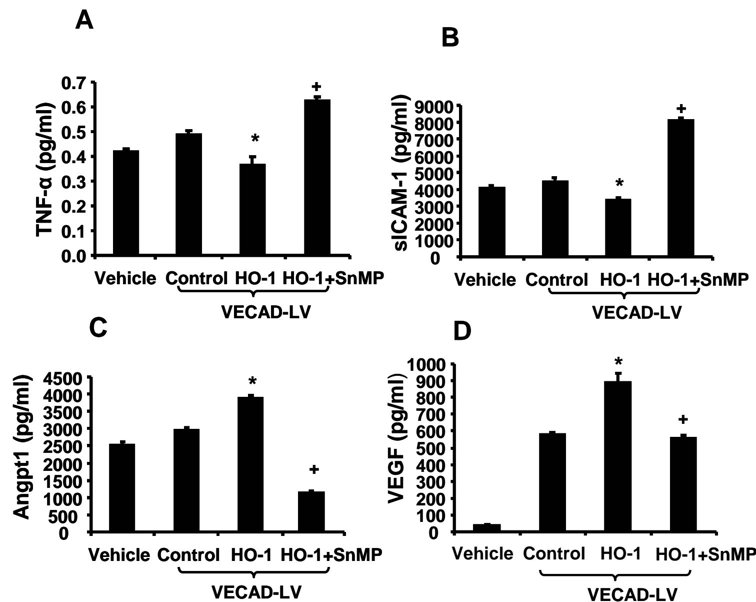

Figure 4. Cytokine array analysis of (A) TNF-α, (B) sICAM-1, (C) Angpt1, and (D) VEGF following treatment of HMEC-1 with VECAD-GFP (control), VECAD-HO-1, and VECAD-HO-1 + SnMP (*$p < 0.05$ vs control, $^{+}p < 0.05$ vs HO-1).

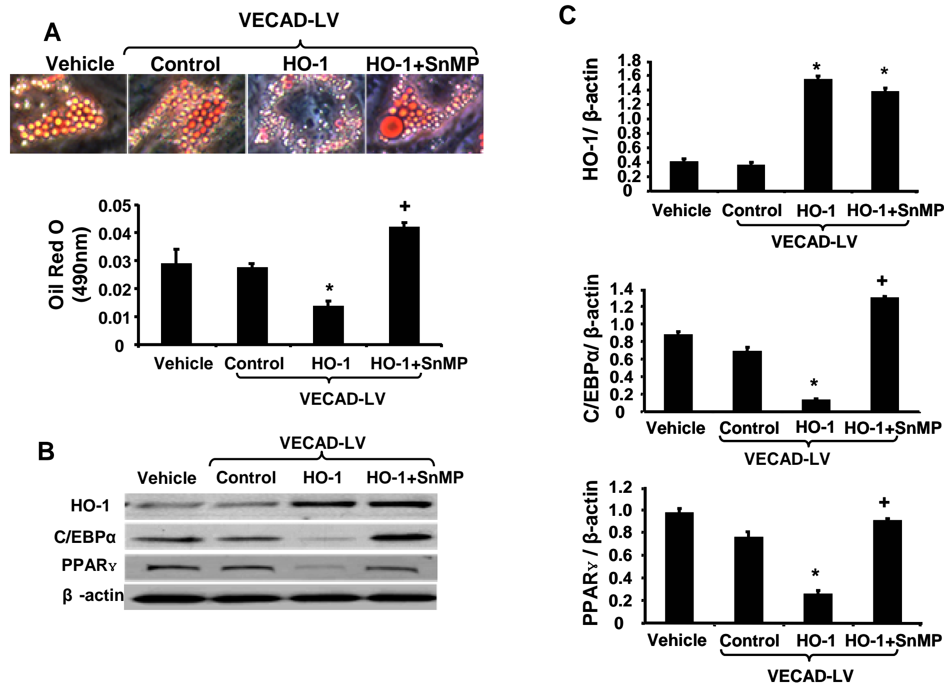

Figure 5. (A) Representative photographs and analysis of adipogenesis in MSC-derived adipocytes following administration of CM from EC treated with VECAD-GFP (control), VECAD-HO-1, and VECAD-HO-1 + SnMP. (B) Representative Western blots and (C) densitometric analysis of HO-1, C/EBP-α, and PPAR-γ expression from MSC-derived adipocytes following administration of CM from EC treated with VECAD-GFP, VECAD-HO-1, and VECAD-HO-1 + SnMP, *$p < 0.05$ vs control, $^{+}p < 0.05$ vs HO-1.

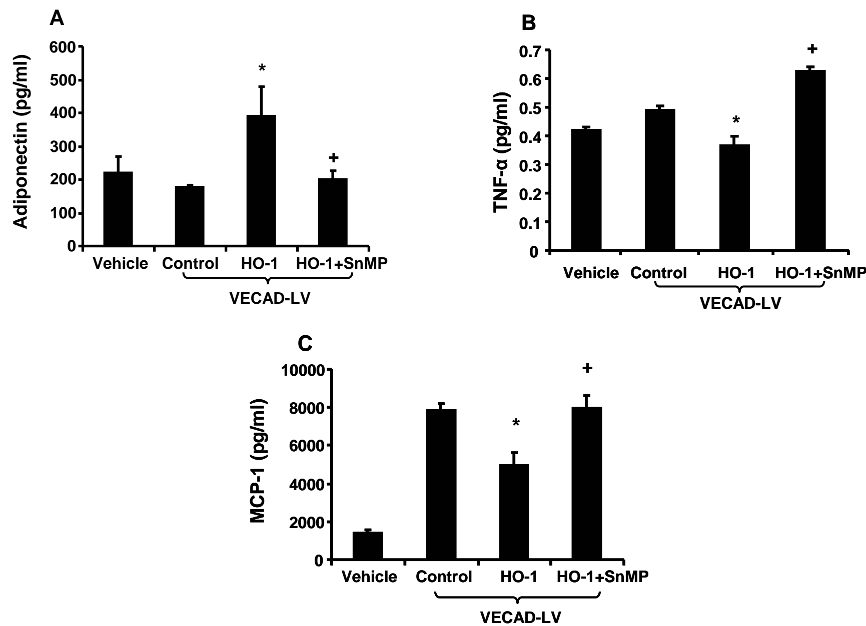

Figure 6. Cytokine array analysis of (A) adiponectin, (B) TNF-α, and (C) MCP-1 from MSC-derived adipocytes following administration of CM from EC treated with VECAD-GFP (Control), VECAD-HO-1, and VECAD-HO-1 + SnMP, *$p < 0.05$ vs Control, $^{+}p < 0.05$ vs HO-1.

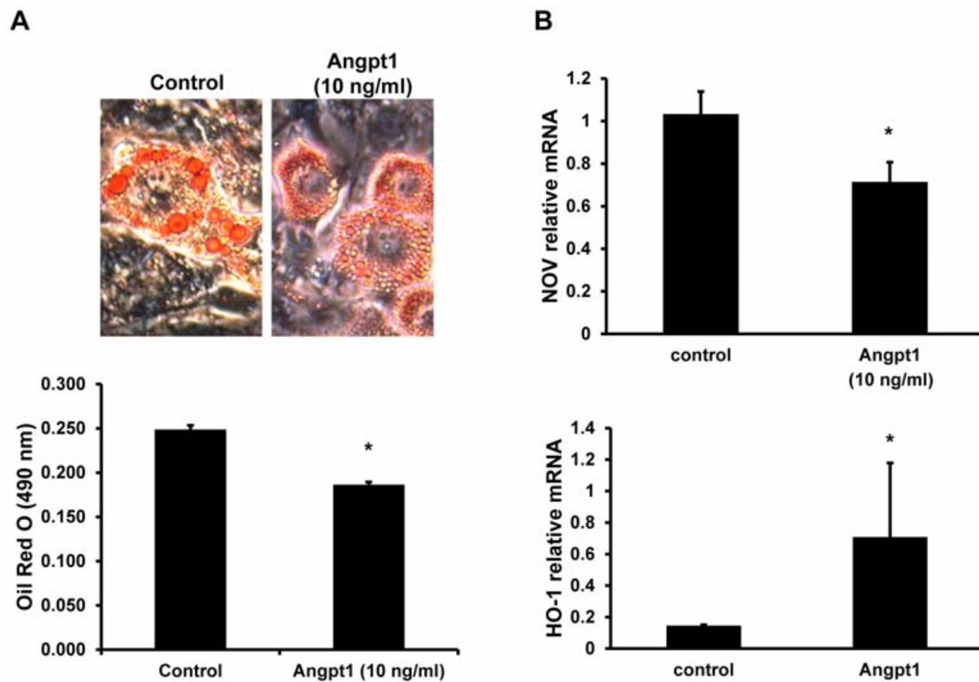

Figure 7. (A) Representative photographs and analysis of adipogenesis in 3T3-L1-derived adipocytes treated with two variable concentrations of Angpt1. (B) NOV mRNA levels in 3T3-L1 cells treated with Angpt1 (10 ng/ml) for 5 days, *p < 0.05 vs control.

VECAD-GFP lentivirus resulted in adipocytes releasing similar levels of adiponectin, TNF-α and MCP-1 as compared to the vehicle. Conditioned medium from EC transduced with VECAD-HO-1 increased the adipocyte CM levels of adiponectin and decreased MCP-1 and TNF-α levels. In contrast, CM from adipocytes treated with CM from EC administered VECAD-HO-1 and SnMP displayed decreased levels of adiponectin, as well as increased levels of MCP-1 and TNF-α (Figure 6A-C).

Effect of Angpt1 on adipogenesis

As shown earlier, Angpt1 and VEGF increased in parallel with the up-regulation of HO-1 gene expression. Therefore, 3T3-L1 pre-adipocytes were treated with these biomarkers in order to examine their effect on adipogenesis independently. Figure 7A reveals that adipogenesis is mitigated in the presence of physiological concentration of Angpt1 (10 ng/ml). Interestingly, as seen in Figure 7B, treatment of 3T3-L1 adipocytes with Angpt1 (10 ng/ml) during differentiation reduced NOV mRNA levels ($p < 0.05$), and increased HO-1 mRNA levels ($p < 0.05$) as compared to control.

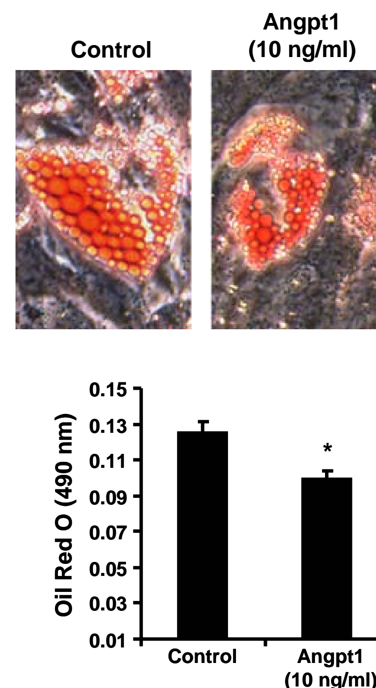

Figure 8. Representative photographs and analysis of adipogenesis in MSC-derived adipocytes treated with two variable concentrations of Angpt1, *p < 0.05 vs control.

To further confirm the Angpt1-mediated inhibition of adipogenesis, MSCs were treated with Angpt1. As expected, treatment of MSCs with Angpt1, inhibited adipogenesis (Figure 8). VEGF (10 and 100 ng/ml) had minimal, if any, effect on adipogenesis in 3T3-L1 pre-adipocytes or MSCs (data not shown).

DISCUSSION

The present report demonstrates that cell-specific targeting of EC with HO-1 was effective in modifying adipogenesis such that smaller, less inflamed adipocytes were produced. Endothelial cells produce positive and negative growth factors that regulate adipogenic hyperplasia and hypertrophy in MSC-derived adipocytes. EC dysfunction leads to the production of negative regulators of adipocyte function, such as the release of TNF-α and MCP-1. Protection of EC by either gene-targeting using VECAD-HO-1 or pharmacologically by CoPP resulted in a reduction in EC ICAM-1 levels, as well as a decrease in the release of inflammatory markers TNF-α and MCP-1 from adipocytes. Increased HO-1 levels in EC also resulted in a significant increase in Angpt1 from EC, along with an increase in adiponectin from adipocytes, ultimately leading to smaller, healthier adipocytes. The beneficial effect of Angpt1 was confirmed as adipogenesis was reduced in cells treated with Angpt1. Angpt1 treatment also reduced the expression of the proinflammatory adipokine, NOV in adipocytes. Together, these results demonstrate that functional EC promote healthy adipocytes and suppress adipocyte-mediated release of inflammatory cytokines. Several key findings substantiate this conclusion: first, Angpt1 has been shown to improve lipolytic activity and to increase insulin sensitivity *in vitro* [32] and also to inhibit MSC differentiation into adipocytes [33]. However, treatment of EC with AngII stimulates endothelial dysfunction by activation of NADPH oxidase [66]. This was confirmed by increased production of ICAM-1 in AngII-treated EC. The role of ICAM-1 in experimental atherosclerosis and type II diabetes mellitus, as a leukocyte adhesion receptor in regions of inflammation, is well established [67, 68]. In addition, previous *in vivo* studies have implicated ICAM-1 in the development of central obesity [69]. Treatment of MSCs with CM from AngII-treated EC possessing elevated levels of ICAM-1 resulted in an increase in adipogenesis and cell size of MSC-derived adipocytes. This effect was blocked in CM from EC in which HO-1 expression was increased. Several studies have demonstrated that induction of HO-1 suppresses NADPH oxidase-induced oxidative stress [70-72]. Thus, the HO system serves a key protective role in the cardiovascular system [44] against the harmful effects of AngII-induced oxidative stress by regulating inflammatory factors such as ICAM-1 [73-75]. HO-1 induction in EC resulted in a decrease in ICAM-1 levels and decreased adipogenesis in MSCs, confirming the existence of a link between vasculature homeostasis and adipose tissue function.

Secondly, our results demonstrate that transduction with VECAD-HO-1 improves EC function by reducing the expression of inflammatory markers. The beneficial effect of HO-1 was negated by the inhibitor of HO-activity, SnMP [76]. This confirms that increased HO activity is responsible for the reduction in inflammatory agents within EC. In order to understand the potential mediators of perivascular communication between EC and adipocytes *in vitro*, we studied the effects of HO induction on several biomarkers in the CM of the EC treated with our VECAD-HO-1 virus. TNF-α and sICAM-1 levels were reduced in CM of cells transduced with VECAD-HO-1 while the levels of Angpt1 and VEGF were elevated. Given that administration of exogenous Angpt1 has previously been demonstrated to result in a decrease in lipid droplet diameter, this data suggests that EC-derived Angpt1 may be a major negative regulator of adipogenesis [31-33]. Similar to Angpt1, VEGF induces angiogenesis as a means of restoring oxygen levels to hypoxic cells. As adipose tissue expands in obesity, cell size grows and increased angiogenesis is necessary to provide adequate nutrients to these inflamed adipocytes; otherwise, hypoxia develops, leading to changes in cytokine expression, pro-inflammatory recruitment, and insulin resistance [30, 77-79]. Angpt1 and VEGF promote adipose tissue angiogenesis and slow the development of unhealthy adipose tissue. Conditioned medium collected from EC treated with VECAD-HO-1 had higher expression of Angpt1 and VEGF than the control, indicating inhibition of adipogenesis and a decreased risk of hypoxia-induced inflammation and oxidative stress in MSC-derived adipocytes [30, 80, 81].

Our results provide evidence that alteration in endothelial-adipocyte cell communication can modify adipocyte function and contribute to the development of obesity. Adipocyte size is a reflection of adipocyte function, e.g., small adipocytes are healthy and secrete more adiponectin and lower levels of adipocytokines than larger, inflamed adipocytes [55]. Adiponectin is an important indicator of adipocyte health and it is one of very few innate insulin sensitizers [82]. Low plasma concentration of adiponectin has a strong correlation to adipocyte dysfunction seen in diabetes mellitus type 2 (DM2) and obesity. C/EBP-α and PPAR-γ, activate inflammatory cytokines such as MCP-1 in the adipocyte inflammatory pathway and ultimately affect adipocyte size [83, 84, 85]. MSCs grown in VECAD-HO-1-treated EC CM displayed a dramatic depression in C/EBP-α and PPAR-γ levels when compared to control. On the other hand, MSCs cultured with CM from (VECAD-HO-1 transduced + SnMP) EC exhibited elevated levels of both transcription factors. Adipocytes derived from EC CM treated with VECAD-HO-1 had a two-fold higher concentration of adiponectin than the control, and these adipocytes were small and healthy. Furthermore, MCP-1 and TNF-α secretion was decreased in adipocytes with a high adiponectin concentration, providing further proof of perivascular communication between adipose tissue and nearby vessels.

CONCLUSION

Our data confirms the existence of crosstalk between EC and perivascular adipose tissue. We demonstrate that overexpression of endothelial HO-1 significantly inhibits adipogenesis. Up-regulation of HO-1 in EC also resulted in significant decreases in inflammatory markers and substantial increases in Angpt1 and VEGF, which may mediate downstream inhibition of adipogenesis by EC overexpression of HO-1. These results suggest that alterations in EC function often associated with obesity can directly modify adipocyte differentiation, increase the release of adipocytokines, and promote inflammation in obesity. Moreover, our results demonstrate that targeting of EC with HO-1 to reduce endothelial inflammation has significant effects on the differentiation of MSCs to adipocytes and can increase the production of beneficial adipokines such as adiponectin. Further studies are required to fully elucidate the therapeutic potential of this newly described crosstalk between EC and perivascular adipose tissue.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Abraham, N. G. and Kappas, A. 2008, Pharmacol. Rev., 60, 79-127.
2. Luo, C., Lv, N., Chang, Z., Qu, Q. and Huang, J. 2017, Eur. J. Cell Biol., 96, 567-578.
3. Newsholme, P., Cruzat, V. F., Keane, K. N., Carlessi, R. and de Bittencourt, P. I. 2016, Biochem. J., 473, 4527-4550.
4. Bhattacharya, I. and Ullrich, A. 2006, FEBS Lett., 580, 5765-5771.
5. Gu, P. and Xu, A. 2013, Rev. Endocr. Metab. Disord., 14, 49-58.
6. Li, M., Qian, M. and Xu, J. 2017, Front Cardiovasc. Med., 4, 51.
7. Matsumoto, T., Kobayashi, T. and Kamata, K. 2008, J. Smooth Muscle Res., 44, 41-55.
8. Wang, N., Verna, L., Chen, N. G., Chen, J., Li, H., Forman, B. M. and Stemerman, M. B. 2002, J. Biol. Chem., 277, 34176-34181.
9. Yubero-Serrano, E. M., Delgado-Lista, J., Peña-Orihuela, P., Perez-Martinez, P., Fuentes, F., Marin, C., Tunez, I., Tinahones, F. J., Perez-Jimenez, F., Roche, H. M. and Lopez-Miranda, J. 2013, Exp. Mol. Med., 45, e28.
10. Schling, P. and Schäfer, T. 2002, J. Biol. Chem., 277, 48066-48075.
11. Sodhi, K., Puri, N., Kim, D. H., Hinds, T. D., Stechschulte, L. A., Favero, G., Rodella, L., Shapiro, J. I., Jude, D. and Abraham, N. G.. 2014, Int. J. Obes[Lond], 38, 456-465.

12. Tsukuda, K., Mogi, M., Iwanami, J., Kanno, H., Nakaoka, H., Wang, X. L., Bai, H. Y., Shan, B. S., Kukida, M., Higaki, A., Yamauchi, T., Min, L. J. and Horiuchi, M. 2016, PLoS One, 11, e0167704.
13. Griendling, K. K., Minieri, C. A., Ollerenshaw, J. D. and Alexander, R. W. 1994, Circ. Res., 74, 1141-1148.
14. Cassis, L. A., Police, S. B., Yiannikouris, F. and Thatcher, S. E. 2008, Curr. Hypertens. Rep., 10, 93-98.
15. Furuhashi, M., Ura, N., Higashiura, K., Murakami, H., Tanaka, M., Moniwa, N., Yoshida, D. and Shimamoto, K. 2003, Hypertension, 42, 76-81.
16. Ford, E. S. 2005, Diabetes Care, 28, 2745-2749.
17. Hall, J. E., Brands, M. W., Dixon, W. N. and Smith, M. J. 1993, Hypertension, 22, 292-299.
18. Fontana, L., Eagon, J. C., Trujillo, M. E., Scherer, P. E. and Klein, S. 2007, Diabetes, 56, 1010-1013.
19. Tang, J., Yan, H. and Zhuang, S. 2012, Int. J. Nephrol., 2012, 608397.
20. Jones, J. R., Barrick, C., Kim, K. A., Lindner, J., Blondeau, B., Fujimoto, Y., Shiota, M., Kesterson, R. A., Kahn, B. B. and Magnuson, M. A. 2005, Proc. Natl. Acad. Sci. USA, 102, 6207-6212.
21. Kintscher, U. and Law, R. E. 2005, Am. J. Physiol. Endocrinol. Metab., 288, E287-291.
22. Li, J. J., Wang, R., Lama, R., Wang, X., Floyd, Z. E., Park, E. A. and Liao, F. F. 2016, Sci. Rep., 6, 38550.
23. Wang, F., Mullican, S. E., DiSpirito, J. R., Peed, L. C. and Lazar, M. A. 2013, Proc. Natl. Acad. Sci. USA, 110, 18656-18661.
24. Duan, S. Z., Usher, M. G. and Mortensen, R. M. 2008, Circ. Res., 102, 283-294.
25. Aicher, A., Zeiher, A. M. and Dimmeler, S. 2005, Hypertension, 45, 321-325.
26. McClung, J. M., Reinardy, J. L., Mueller, S. B., McCord, T. J., Kontos, C. D., Brown, D. A., Hussain, S. N., Schmidt, C. A., Ryan, T. E. and Green, T. D. 2015, Front Physiol., 6, 161.
27. Nykänen, A. I., Krebs, R., Saaristo, A., Turunen, P., Alitalo, K., Ylä-Herttuala, S., Koskinen, P. K. and Lemström, K. B. 2003, Circulation, 107, 1308-1314.
28. Tomada, N., Tomada, I., Botelho, F., Pacheco-Figueiredo, L., Lopes, T., Negrão, R., Pestana, M. and Cruz, F. 2013, Andrology, 1, 541-548.
29. Chislock, E. M., Ring, C. and Pendergast, A. M. 2013, Proc. Natl. Acad. Sci. USA, 110, 12432-12437.
30. Dallabrida, S. M., Zurakowski, D., Shih, S. C., Smith, L. E., Folkman, J., Moulton, K. S. and Rupnick, M. A. 2003, Biochem. Biophys. Res. Commun., 311, 563-571.
31. Jung, Y. J., Choi, H. J., Lee, J. E., Lee, A. S., Kang, K. P., Lee, S., Park, S. K., Park, T. S., Jin, H. Y., Lee, S. Y., Kim, D. H. and Kim, W. 2012, Biochem. Biophys. Res. Commun., 420, 498-504.
32. Pellegrinelli, V., Rouault, C., Veyrie, N., Clément, K. and Lacasa, D. 2014, Diabetes, 63, 535-549.
33. Kim, S., Lee, J. C., Cho, E. S. and Kwon, J. 2013, J. Orthop. Res., 31, 1920-1928.
34. Cao, J., Singh, S. P., McClung, J. A., Joseph, G., Vanella, L., Barbagallo, I., Jiang, H., Falck, J. R., Arad, M., Shapiro, J. I. and Abraham, N. G. 2017, Am. J. Physiol. Heart Circ. Physiol., 313, H368-H380.
35. Martinerie, C., Garcia, M., Do, T. T., Antoine, B., Moldes, M., Dorothee, G., Kazazian, C., Auclair, M., Buyse, M., Ledent, T., Marchal, P. O., Fesatidou, M., Beisseiche, A., Koseki, H., Hiraoka, S., Chadjichristos, C. E., Blondeau, B., Denis, R. G., Luquet, S. and Fève, B. 2016, Diabetes, 65, 2502-2515.
36. Pakradouni, J., Le Goff, W., Calmel, C., Antoine, B., Villard, E., Frisdal, E., Abifadel, M., Tordjman, J., Poitou, C., Bonnefont-Rousselot, D., Bittar, R., Bruckert, E., Clément, K., Fève, B., Martinerie, C. and Guérin, M. 2013, PLoS One, 8, e66788.
37. Singh, S. P., Grant, I., Meissner, A., Kappas, A. and Abraham, N. G. 2017, Horm. Mol. Biol. Clin. Investig., 31, 2017.
38. Burgess, A., Li, M., Vanella, L., Kim, D. H., Rezzani, R., Rodella, L., Sodhi, K., Canestraro, M., Martasek, P., Peterson, S. J., Kappas, A. and Abraham, N. G. 2010, Hypertension, 56, 1124-1130.
39. Singh, S. P., Bellner, L., Vanella, L., Cao, J., Falck, J. R., Kappas, A. and Abraham, N. G. 2016, J. Nutr. Metab., 2016, 9039754.

40. Singh, S. P., Schragenheim, J., Cao, J., Falck, J. R., Abraham, N. G. and Bellner, L. 2016, Prostaglandins Other Lipid Mediat., 125, 8-18.
41. van den Berghe, N., Ouwens, D. M., Maassen, J. A., van Mackelenbergh, M. G., Sips, H. C. and Krans, H. M. 1994, Mol. Cell Biol., 14, 2372-2377.
42. Waldman, M., Bellner, L., Vanella, L., Schragenheim, J., Sodhi, K., Singh, S. P., Lin, D., Lakhkar, A., Li, J., Hochhauser, E., Arad, M., Darzynkiewicz, Z., Kappas, A. and Abraham, N. G. 2016, Stem Cells Dev., 25, 1084-1094.
43. Di Noia, M. A., van Driesche, S., Palmieri, F., Yang, L. M., Quan, S., Goodman, A. I. and Abraham, N. G. 2006, J. Biol. Chem., 281, 15687-15693.
44. Stec, D. E., Drummond, H. A., Gousette, M. U., Storm, M. V., Abraham, N. G. and Csongradi, E. 2012, J. Am. Soc. Nephrol., 23, 834-841.
45. Abraham, N. G., Junge, J. M. and Drummond, G. S. 2016, Trends Pharmacol. Sci., 37(1), 17-36.
46. Romashko, M., Schragenheim, J., Abraham, N. G. and McClung, J. A. 2016, Trends Pharmacol. Sci., 37, 945-962.
47. Eisenstein, R. S., Garcia-Mayol, D., Pettingell, W. and Munro, H. N. 1991, Proc. Natl. Acad. Sci. USA, 88, 688-692.
48. Ollinger, R., Bilban, M., Erat, A., Froio, A., McDaid, J., Tyagi, S., Csizmadia, E., Graça-Souza, A. V., Liloia, A., Soares, M. P., Otterbein, L. E., Usheva, A., Yamashita, K. and Bach, F. H. 2005, Circulation, 112, 1030-1039.
49. Ollinger, R., Yamashita, K., Bilban, M., Erat, A., Kogler, P., Thomas, M., Csizmadia, E., Usheva, A., Margreiter, R. and Bach, F. H. 2007, Cell Cycle, 6, 39-43.
50. Suematsu, M., Kashiwagi, S., Sano, T., Goda, N., Shinoda, Y. and Ishimura, Y. 1994, Biochem. Biophys. Res. Commun., 205, 1333-1337.
51. Chang, S. H., Barbosa-Tessmann, I., Chen, C., Kilberg, M. S. and Agarwal, A. 2002, J. Biol. Chem., 277, 1933-1940.
52. Quan, S., Kaminski, P. M., Yang, L., Morita, T., Inaba, M., Ikehara, S., Goodman, A. I., Wolin, M. S. and Abraham, N. G. 2004, Biochem. Biophys. Res. Commun., 315, 509-516.
53. L'Abbate, A., Neglia, D., Vecoli, C., Novelli, M., Ottaviano, V., Baldi, S., Barsacchi, R., Paolicchi, A., Masiello, P., Drummond, G. S., McClung, J. A. and Abraham, N. G. 2007, Am. J. Physiol. Heart Circ. Physiol., 293, H3532-3541.
54. Turer, A. T. and Scherer, P. E. 2012, Diabetologia, 55, 2319-2326.
55. Asterholm, I. W. and Scherer, P. E. 2010, Am. J. Pathol., 176, 1364-1376.
56. Vernochet, C., Davis, K. E., Scherer, P. E. and Farmer, S. R. 2010, Endocrinology, 151, 586-594.
57. Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., Mori, Y., Ide, T., Murakami, K., Tsuboyama-Kasaoka, N., Ezaki, O., Akanuma, Y., Gavrilova, O., Vinson, C., Reitman, M. L., Kagechika, H., Shudo, K., Yoda, M., Nakano, Y., Tobe, K., Nagai, R., Kimura, S., Tomita, M., Froguel, P. and Kadowaki, T. 2001, Nat. Med., 7, 941-946.
58. Friedman, J. M. 2011, Keio J. Med., 60, 1-9.
59. Olszanecki, R., Rezzani, R., Omura, S., Stec, D. E., Rodella, L., Botros, F. T., Goodman, A. I., Drummond, G. and Abraham, N. G. 2007, Am. J. Physiol. Renal. Physiol., 292, F148-157.
60. Sabaawy, H. E., Zhang, F., Nguyen, X., ElHosseiny, A., Nasjletti, A., Schwartzman, M., Dennery, P., Kappas, A. and Abraham, N. G. 2001, Hypertension, 38, 210-215.
61. Yang, L., Quan, S. and Abraham, N. G. 1999, Am. J. Physiol., 277, L127-133.
62. Yang, L., Quan, S., Nasjletti, A., Laniado-Schwartzman, M. and Abraham, N. G. 2004, Hypertension, 43, 1221-1226.
63. Asija, A., Peterson, S. J., Stec, D. E. and Abraham, N. G. 2007, Antioxid Redox Signal, 9, 2065-2074.
64. Cao, J., Sodhi, K., Inoue, K., Quilley, J., Rezzani, R., Rodella, L., Vanella, L., Germinario, L., Stec, D. E., Abraham, N. G. and Kappas, A. 2011, Hum. Gene. Ther., 22, 271-282.
65. Li, M., Kim, D. H., Tsenovoy, P. L., Peterson, S. J., Rezzani, R., Rodella, L. F., Aronow, W. S., Ikehara, S. and Abraham, N. G. 2008, Diabetes, 57, 1526-1535.
66. Nakao, T., Morita, H., Maemura, K., Amiya, E., Inajima, T., Saito, Y., Watanabe, M., Manabe, I., Kurabayashi, M., Nagai, R. and Komuro, I. 2013, J. Pineal. Res., 55, 287-293.

67. Isoppo de Souza, C., Rosa, D. D., Ettrich, B., Cibeira, G. H., Giacomazzi, J., Tusset, P., Ashton-Prolla, P., Medeiros, L. R., Caleffi, M., Neto, E. C., Moriguchi, E. H. and Graudenz, M. S. 2012, Nutr. Metab. [Lond.], 9, 97.
68. Kitagawa, K., Matsumoto, M., Sasaki, T., Hashimoto, H., Kuwabara, K., Ohtsuki, T. and Hori, M. 2002, Atherosclerosis, 160, 305-310.
69. Brake, D. K., Smith, E. O., Mersmann, H., Smith, C. W. and Robker, R. L. 2006, Am. J. Physiol. Cell Physiol., 291, C1232-1239.
70. Datla, S. R., Dusting, G. J., Mori, T. A., Taylor, C. J., Croft, K. D. and Jiang, F. 2007, Hypertension, 50, 636-64.
71. Sodhi, K., Hilgefort, J., Banks, G., Gilliam, C., Stevens, S., Ansinelli, H. A., Getty, M., Abraham, N. G., Shapiro, J. I. and Khitan, Z. 2016, Stem Cells Int., 2016, 8197325.
72. Wolin, M. S. and Abraham, N. G. 2006, Hypertension, 48, 826-827.
73. Kim, D. H., Burgess, A. P., Li, M., Tsenovoy, P. L., Addabbo, F., McClung, J. A., Puri, N. and Abraham, N. G. 2008, J. Pharmacol. Exp. Ther., 325, 833-840.
74. Pflueger, A., Croatt, A. J., Peterson, T. E., Smith, L. A., d'Uscio, L. V., Katusic, Z. S. and Nath, K. A. 2005, Am. J. Physiol. Renal. Physiol., 288, F552-558.
75. Vanella, L., Sodhi, K., Kim, D. H., Puri, N., Maheshwari, M., Hinds, T. D., Bellner, L., Goldstein, D., Peterson, S. J., Shapiro, J. I. and Abraham, N. G. 2013, Stem Cell Res. Ther., 4, 28.
76. Abate, A., Zhao, H., Wong, R. J. and Stevenson, D. K. 2007, Biochem. Biophys. Res. Commun., 354, 757-763.
77. Elias, I., Franckhauser, S., Ferré, T., Vilà, L., Tafuro, S., Muñoz, S., Roca, C., Ramos, D., Pujol, A., Riu, E., Ruberte, J. and Bosch, F. 2012, Diabetes, 61, 1801-1813.
78. Hoeben, A., Landuyt, B., Highley, M. S., Wildiers, H., van Oosterom, A. T. and De Bruijn, E. A. 2004, Pharmacol. Rev., 56, 549-580.
79. Loboda, A., Jazwa, A., Wegiel, B., Jozkowicz, A. and Dulak, J. 2005, Cell Mol. Biol. [Noisy-le-grand], 51, 347-355.
80. Berendsen, A. D. and Olsen, B. R. 2015, J. Intern. Med., 277, 674-680.
81. Hattori, K., Sumi, T., Yasui, T., Morimura, M., Nobeyama, H., Okamoto, E., Noriyuki, M., Honda, K., Kiyama, H. and Ishiko, O. 2004, Int. J. Mol. Med., 13, 395-399.
82. Lin, H. V., Kim, J. Y., Pocai, A., Rossetti, L., Shapiro, L., Scherer, P. E. and Accili, D. 2007, Diabetes, 56, 1969-1976.
83. Hu, H. M., Tian, Q., Baer, M., Spooner, C. J., Williams, S. C., Johnson, P. F. and Schwartz, R. C. 2000, J. Biol. Chem., 275, 16373-16381.
84. Ji, J. D., Cheon, H., Jun, J. B., Choi, S. J., Kim, Y. R., Lee, Y. H., Kim, T. H., Chae, I. J., Song, G. G., Yoo, D. H., Kim, S. Y. and Sohn, J. 2001, J. Autoimmun., 17, 215-221.
85. Rosen, E. D., Hsu, C. H., Wang, X., Sakai, S., Freeman, M. W., Gonzalez, F. J. and Spiegelman, B. M. 2002, Genes Dev., 16, 22-26.