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REVIEW

The role of perivascular adipose tissue in vascular smooth muscle cell growth

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Adipose tissue is the largest endocrine organ, producing various adipokines and many other substances. Almost all blood vessels are surrounded by perivascular adipose tissue (PVAT), which has not received research attention until recently. This review will discuss the paracrine actions of PVAT on the growth of underlying vascular smooth muscle cells (VSMCs). PVAT can release growth factors and inhibitors. Visfatin is the first identified growth factor derived from PVAT. Decreased adiponectin and increased tumour necrosis factor-α in PVAT play a pathological role for neointimal hyperplasia after endovascular injury. PVAT-derived angiotensin II, angiotensin 1–7, reactive oxygen species, complement component 3, NO and H_2S have a paracrine action on VSMC contraction, endothelial or fibroblast function; however, their paracrine actions on VSMC growth remain to be directly verified. Factors such as monocyte chemoattractant protein-1, interleukin-6, interleukin-8, leptin, resistin, plasminogen activator inhibitor type-1, adrenomedullin, free fatty acids, glucocorticoids and sex hormones can be released from adipose tissue and can regulate VSMC growth. Most of them have been verified for their secretion by PVAT; however, their paracrine functions are unknown. Obesity, vascular injury, aging and infection may affect PVAT, causing adipocyte abnormality and inflammatory cell infiltration, inducing imbalance of PVAT-derived growth factors and inhibitors, leading to VSMC growth and finally resulting in development of proliferative vascular disease, including atherosclerosis, restenosis and hypertension. In the future, using cell-specific gene interventions and local treatments may provide definitive evidence for identification of key factor(s) involved in PVAT dysfunction-induced vascular disease and thus may help to develop new therapies.

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Abbreviations
CAD, coronary artery disease; PVAT, perivascular adipose tissue; VSMC, vascular smooth muscle cell

Introduction

Obesity, defined as excess body fat, is epidemic (Ogden *et al.*, 2006) and harmful to health because it increases the risk of cardiovascular disease, type 2 diabetes and some cancers (Wisse *et al.*, 2007). Adipose tissue is far from being considered simply a passive reservoir for energy storage. Instead, adipose tissue is the largest endocrine organ (Halvorsen *et al.*, 2000; Kahn, 2008) that can produce and secrete many substances, such as various proteins/peptides termed adipokines, free fatty acids (FFA) and steroid hormones, which have essential roles in energy homeostasis, glucose and lipid metabolism, cell viability, control of feeding, thermogenesis, neuroendocrine function, reproduction, immunity and cardiovascular function (Flier, 2004; Kershaw and Flier, 2004). Most research so far has focused on the endocrine roles of those substances in the development of vascular disease without considering the paracrine role of the adipose tissue surrounding the blood vessels. In fact, blood vessels are composed of three layers: intima, media and adventitia (Figure 1).

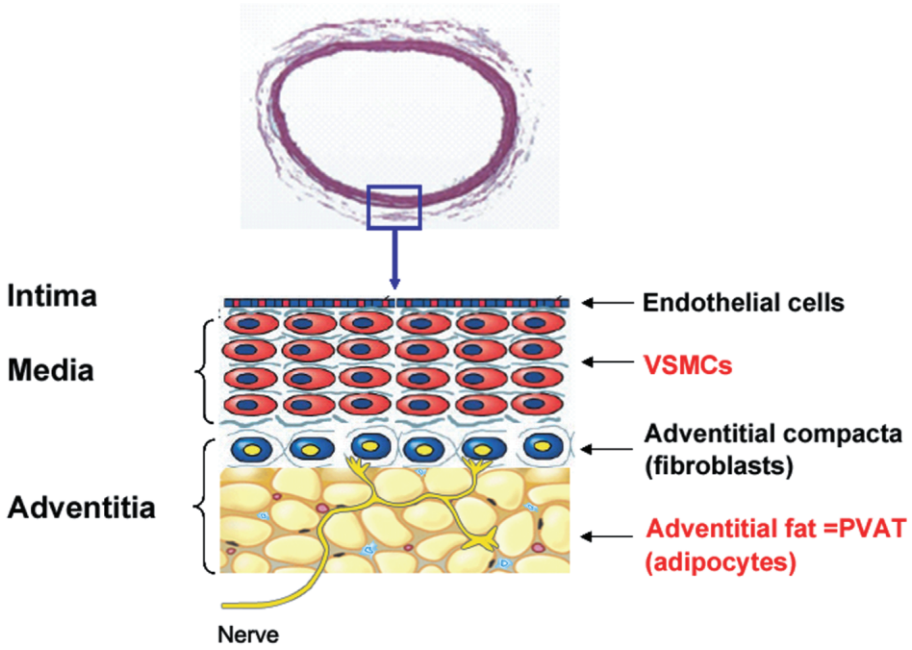

Figure 1

Vascular layer structure. In the PVAT, the yellow, red and blue, respectively, indicate adipocytes, vasa vasorum and other cells (macrophages, adipocyte stem/progenitor cells, lymphocytes, fibroblasts, etc.).

The adventitia harbouring nerve endings can be divided into two sub-layers: adventitial compacta containing as main cell-type fibroblasts and adventitial fat containing as main cell-type adipocytes. Adventitial fat is also called perivascular fat or perivascular adipose tissue (PVAT); it surrounds almost all blood vessels except the cerebral vasculature (Gao, 2007). Traditionally, the study of vascular function regulation has been based on layer-specific mechanisms. The intimal endothelial cells can produce and release a variety of vasoactive substances to modulate medial vascular smooth muscle cell (VSMC) function (Busse *et al.*, 2002; Miao *et al.*, 2005; Feletou *et al.*, 2008). The nerves play an important role in the regulation of medial function (Gutterman, 1999). And the adventitial compacta may also regulate medial function (Gonzalez *et al.*, 2001; Sartore *et al.*, 2001; Somoza *et al.*, 2005). However, PVAT had long been thought to provide only structural support for the blood vessel and has been routinely removed in the traditional isolated blood vessel studies.

Given that adipocytes can secrete numerous active substances, and since perivascular adipocytes encroach into the adventitial compacta without an anatomical barrier, mediators secreted by PVAT may readily gain access into the blood vessel wall, and PVAT may function as a paracrine organ that transduces metabolic signals to blood vessels (Rajsheker *et al.*, 2010). However, little attention had been paid to this possibility until Soltis and Cassis demonstrated that PVAT attenuates the contractile response to norepinephrine in the rat aorta (Soltis and Cassis, 1991), and Lohn *et al.* demonstrated that PVAT releases an unknown relaxing factor (Lohn *et al.*, 2002). In 2005, Barandier *et al.* demonstrated that PVAT secreted growth factors, which can stimulate the proliferation of VSMCs, providing a new notion that PVAT plays roles in not only vascular tone but also other biological properties of VSMCs and thus opening new perspectives in vascular biology. Thereafter, we identified visfatin, an adipokine, as a PVAT-derived growth factor for VSMCs (Wang *et al.*, 2009). Most recently, *in vivo* experiments demonstrated that implanting adipose tissue to surround the blood vessel may affect neointimal formation following endovascular injury, indicating the role of PVAT in VSMC growth and migration (Takaoka *et al.*, 2009). The vascular surrounding tissue (PVAT mainly) was also pointed out being important for saphenous vein graft patency in pioneer human studies (Souza *et al.*, 2001; Souza *et al.*, 2006). Here, we will review the influence of PVAT on VSMC growth and migration and discuss its possible role and potential therapy in proliferative vascular diseases.

PVAT structure and heterogeneity

Two types of adipose tissue exist in the body: brown adipose tissue as non-shivering thermogenerator and white adipose tissue as depository for excessive energy. They are endowed with distinct vascular and nervous supplies: the brown adipose tissue has a greater density of capillaries and noradrenergic fibres than white adipose tissue. PVAT, like other adipose depots, has a mixed composition of both types of adipose tissue, with different white/brown adipose tissue ratio in different organs (Cinti, 2011). In Sv129 mice, the mediastinal depot (coronary artery and thoracic aorta) consists mainly of brown adipose tissue, the abdomino-pelvic depot (iliofemoral vessels) is equally composed of white and brown adipose tissue, while the retroperitoneal and mesenteric depots (abdominal aorta and mesenteric artery) contain

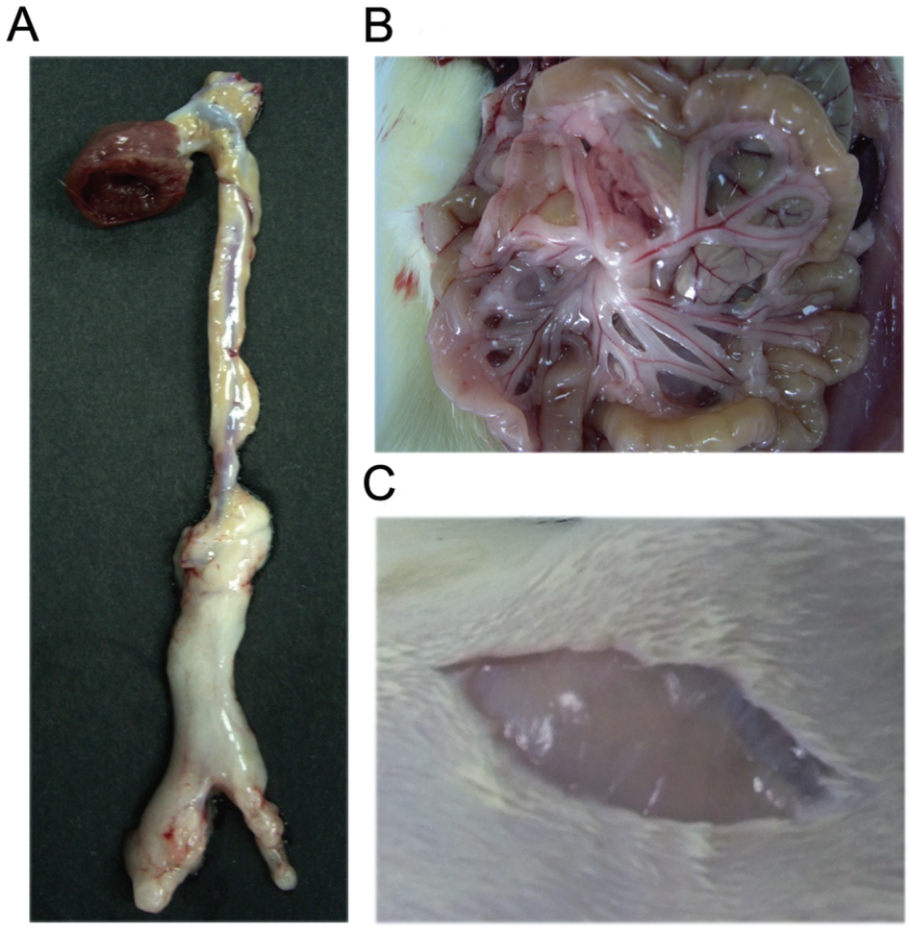

Figure 2

Photographs of periaortic (A), mesenteric (B) and inguinal subcutaneous (C) adipose tissue in Sprague–Dawley rats.

mainly white adipose tissue (Frontini and Cinti, 2010; Cinti, 2011). Our results showed that in Sprague–Dawley rats, PVAT surrounding thoracic aorta is mainly brown adipose tissue (Wang *et al.*, 2009). Moreover, it is obviously different from the PVAT surrounding abdominal aorta (Figure 2A) and mesenteric artery (Figure 2B) and the inguinal subcutaneous adipose tissue (Figure 2C), which are mainly white adipose tissue. In humans, brown adipose tissue is mainly located in PVAT around the aorta and its main branches including carotid, subclavian, intercostal and renal arteries in relation to the need to distribute the heat produced by brown adipose tissue to the body via the circulation (Frontini and Cinti, 2010). The data for human coronary PVAT are conflicting: one study indicates that epicardial adipose tissue harvested from the origin of human right coronary arteries expresses higher levels of brown adipocyte-related genes compared with other regional adipose depots (Sacks *et al.*, 2009), while another study indicates that the gene expression profile in human perivascular adipocytes surrounding coronary arteries reflects white, rather than brown, adipocytes (Chatterjee *et al.*, 2009). This difference may reflect different methods, individual variations or be due to harvesting epicardial adipose tissues from different parts of the coronary arteries. It should be noted that epicardial adipose tissue is a mixture of that surrounding the large coronary vessels but is also present in significant quantities in humans on the surface of the ventricles, especially the right, and the apex of the heart (Sacks and Fain, 2007). There are no obvious anatomical boundaries between coronary PVAT and epicardial adipose tissue in humans. Adipocyte transdifferentiation may occur; in conditions of chronic cold exposure, white-to-brown conversion meets the need for thermogenesis, whereas an obesogenic diet induces brown-to-white conversion to meet the need for storing energy (Cinti, 2011). Species differences may exist; in contrast to humans, little or no epicardial adipose tissue is seen in rats and mice (Marchington *et al.*, 1989).

PVAT is a kind of connective tissue. Besides cell components (mainly adipocytes), it contains collagen and elastic fibres, nerve bundles and vasa vasorum (Lebona, 1993). Adventitial vasa vasorum neovascularization occurs during vascular injury and inflammation, providing a direct way to transmit substances released by PVAT to the inner vasculature (Kwon *et al.*, 1998; Gossl *et al.*, 2009). Luminal terminations of the vasa vasorum in the saphenous vein have been described, and this microvascular network may be a system transporting vasoactive factors within the vascular wall (Dashwood *et al.*, 2007), providing a structural basis for the cell crosstalk between different layers of blood vessel. The cell

types in PVAT include adipocytes, endothelial cells, macrophages, adipocyte stem/progenitor cells, lymphocytes, fibroblasts, etc. The percentage of these cells can change with age, nutritional status and environmental conditions. In obese individuals, the periaortic PVAT has more inflammatory cells infiltrated and is expanding in response to metabolic stimuli (Skilton *et al.*, 2009). Perivascular adipocytes surrounding human coronary arteries are more heterogeneous in shape and smaller in size and exhibit a reduced state of adipogenic differentiation than subcutaneous adipocytes (Chatterjee *et al.*, 2009). Throacic periaortic adipocytes are also much smaller in size as compared with perimesenteric adipocytes in the rat (Galvez-Prieto *et al.*, 2008). The mean adipocyte surface and differentiating preadipocyte density in epicardial adipose tissue near the proximal tract of the right coronary artery obtained from coronary artery disease (CAD) patients are larger and increased, respectively, compared with those of non-CAD patients (Silaghi *et al.*, 2007).

PVAT influences VSMC growth and migration

Extensive plasticity is an inherent property exhibited by fully mature VSMCs, which are ordinarily in a non-proliferative, contractile state, but switch to a proliferative, synthetic phenotype during response to injury (Carmeliet, 2000). VSMC growth (proliferation and/or hypertrophy) and migration play a major role in proliferative vascular diseases, such as atherosclerosis, restenosis, graft vasculopathy and hypertension. Atherosclerosis is the most widely acknowledged example in which VSMC phenotypic switching to proliferation and migration plays a key role. Restenosis at sites of vascular injury following angioplasty is due to intimal hyperplasia, in which VSMC proliferation and VSMC migration from media to intima are involved. The local microenvironment, such as growth factors/inhibitors and mechanical influences, is very important for the phenotypic conversion (Owens *et al.*, 2004). Factors released from perivascular fat may be regulators for VSMC proliferation and migration, to judge from the available direct and indirect evidence.

Direct evidence

PVAT-derived growth factors

Adipocytes promote proliferation in some types of cells (Manabe *et al.*, 2003; Amemori *et al.*, 2007). However, the effect of adipocytes on VSMC growth was unknown until Yang *et al.* (Barandier *et al.*, 2005) cultured VSMCs in conditioned medium from 3T3-L1-derived differentiated adipocytes or from 3T3-L1 preadipocytes. They found that VSMC proliferation can be induced by the conditioned medium from differentiated adipocytes rather than that of preadipocytes. The growth factor(s) present in the conditioned medium from mature adipocytes may be a protein(s), since this proliferation is abolished by proteinase K treatment and not sensitive to phospholipase B (to hydrolyze lysophosphatidic acid). In addition, the protein(s) is (are) resistant to trypsin. Further experiments demonstrated that the adipocyte-derived growth factor(s) is (are) hydrosoluble and present in a fraction of molecular mass larger than 100 kDa. Another study also showed that adipocyte-conditioned media generated from human adipocytes can induce a prominent proliferation and migration of human VSMCs. This effect is enhanced in a synergistic way by the combination with oleic acid, a FFA (Lamers *et al.*, 2011).

Moreover, Yang *et al.* (Barandier *et al.*, 2005) showed that the conditioned medium from rat periaortic adipose tissue can stimulate VSMC proliferation, which is partly reduced by heat and proteinase K, suggesting that a protein component is present in conditioned medium from PVAT and is involved in the proliferation-promoting effect. The activity of PVAT-conditioned medium is also resistant to trypsin. But 40% of the biological activity of PVAT-conditioned medium is retained after treatment with heat plus proteinase K, implying that conditioned medium from PVAT has a more complex feature than that from cultured adipocytes. The difference between cultured 3T3-L1-derived adipocytes and adipocytes in PVAT and heterogeneous cell types in periaortic adipose tissue may contribute to this diversity. It seems that PVAT-derived growth factors are composed of both protein and non-protein components. Of interest, the growth-promoting effect of PVAT is significantly enhanced in aged rats and in high-fat diet-induced obese rats, suggesting that PVAT may be involved in vascular disease associated with aging and obesity (Barandier *et al.*, 2005).

Visfatin

Visfatin is an adipokine discovered in 2005 (Fukuhara *et al.*, 2005). This protein is also known as pre-B cell colony-enhancing factor and identified as nicotinamide phosphoribosyltransferase that biosynthesizes nicotinamide mononucleotide (NMN) from nicotinamide (Wang *et al.*, 2011). As a new adipokine, it is mainly expressed in and secreted from visceral fat as opposed to subcutaneous fat. The circulating visfatin level is increased in obesity (Fukuhara *et al.*, 2005; Sandeep *et al.*, 2007; Filippatos *et al.*, 2008; Wang *et al.*, 2010a).

We demonstrated the expression and secretion of visfatin in aortic PVAT (Wang *et al.*, 2009). Visfatin protein expression in PVAT of the rat thoracic aorta was the highest, 3.7-fold and 1.8-fold higher than that in subcutaneous and visceral adipose tissue respectively. Similar results were obtained in monkey adipose tissues. Moreover, visfatin was detected in PVAT-conditioned medium, indicating that visfatin can be released from PVAT.

To characterize the paracrine function of PVAT-derived visfatin, we tested its role in VSMC contraction and proliferation (Wang *et al.*, 2009). The anti-contractile effect of PVAT was not affected by visfatin-specific antibodies and by the visfatin chemical inhibitor FK866. Exogenous visfatin (up to 50 nM) caused neither relaxation nor contraction in aortae with and without endothelium. These observations imply that PVAT-derived visfatin is not involved in the regulation of vascular tone. However, PVAT-derived visfatin was found to be a VSMC growth factor. PVAT-conditioned medium stimulated VSMC proliferation, which was significantly attenuated by visfatin specific antibodies. Exogenous visfatin stimulated

VSMC proliferation, which also could be totally blocked by the visfatin antibody. In addition, visfatin did not show any proliferative effect on VSMCs during co-incubation with FK866. These results indicate that the proliferative effect of PVAT is partly mediated by visfatin. This was the first report that an adipokine secreted by PVAT could be identified as a growth factor for VSMCs (Wang *et al.*, 2009).

Our further experiments demonstrated that exogenous visfatin at physiological concentrations stimulated VSMC proliferation in a dose- and time-dependent manner via extracellular signal-regulated kinase (ERK) 1/2 and p38 rather than c-Jun N-terminal kinase and phosphatidylinositol 3-kinase/Akt signalling pathways. Visfatin exerted anti-apoptotic effects when VSMC apoptosis was induced by H_2O_2, but not under normal conditions. Insulin receptor knockdown did not affect the response to visfatin. Visfatin acted as a nicotinamide phosphoribosyltransferase to biosynthesize NMN, which mediated proliferative signalling pathways and cell proliferation similar to the visfatin effect (Wang *et al.*, 2009).

Another report focused on the pro-inflammatory effects of exogenous visfatin in VSMCs (Romacho *et al.*, 2009) and showed that visfatin induced inducible nitric oxide synthase (iNOS) in a concentration-dependent manner and elicited a sustained activation of ERK1/2 signalling with induction of NF-κB. Intriguingly, consistent with our observations with exogenous NMN (Wang *et al.*, 2009), the authors found that NMN mimicked NF-κB activation and iNOS induction by visfatin, and that this could be prevented by FK866 (Romacho *et al.*, 2009).

The above two studies may open new areas of research related to the role of visfatin in pathology, as proliferation of and inflammation in VSMCs are closely related to the genesis, development and rupture of atherosclerotic plaques (Hansson, 2005). Regarding the relationship between circulating visfatin and atherosclerosis, the available clinical studies yield different conclusions, reporting presence (Zhong *et al.*, 2008; Kadoglou *et al.*, 2010; Mu *et al.*, 2011) and absence (Kato *et al.*, 2009; Rho *et al.*, 2010) of correlation. Local PVAT visfatin was also studied in atherosclerotic patients. Epicardial adipose tissue thickness was correlated with plasma visfatin levels and local visfatin expression in epicardial adipose tissue adjacent to the proximal right coronary artery was increased in CAD patients (Cheng *et al.*, 2008). Moreover, aortic and coronary atherosclerosis was positively correlated with visfatin expression in the corresponding PVAT (Spiroglou *et al.*, 2010). In obesity, visfatin may be increased in systemic circulation and local PVAT. This elevated visfatin may play a role in certain types of atherosclerosis, for example, obesity-related atherosclerosis.

When considering the potential role of visfatin in atherosclerosis, it should be taken into account that it is a multifunctional protein. Visfatin has potent pro-survival effects on the three most important cell types (macrophages, VSMCs and endothelial cells) involved in atherosclerosis, implying a protective role of the adipokine in these cells (van der Veer *et al.*, 2007; Li *et al.*, 2008; Borradaile and Pickering, 2009; Ho *et al.*, 2009; Wang *et al.*, 2009). Together with the fact that dysfunction or death of macrophages, VSMCs and endothelial cells are believed to be critical processes in the genesis and development of atherosclerosis, it remains uncertain whether visfatin is friend or foe.

Adiponectin

Adiponectin is an adipocyte-specific adipokine, independently characterized in 1995 and 1996 by four groups using different methods, hence its alternative names of apM1, Acrp30, adipoQ and GBP28 (Scherer *et al.*, 1995; Hu *et al.*, 1996; Maeda *et al.*, 1996; Nakano *et al.*, 1996). Adiponectin expression is higher in human subcutaneous than visceral adipose tissue (Fain *et al.*, 2004). Circulating adiponectin levels decline in obesity, suggesting that hypoadiponectinemia contributes to the pathological conditions associated with overweight. Most of the available information indicates that adiponectin is a beneficial adipokine having anti-diabetic, anti-inflammatory and anti-atherogenic effects (Matsuzawa, 2010).

Adiponectin is a vasodilator substance but is not involved in the modulation of vascular tone by PVAT (Fesus *et al.*, 2007). However, it has been identified as responsible for the protection by PVAT against neointimal formation after vascular injury (Takaoka *et al.*, 2009). This study provided *in vivo* data supporting the paracrine effect of PVAT on VSMC growth and migration. The authors developed a novel mouse model replacing PVAT with exogenous fat after endovascular wire injury. In the femoral artery, PVAT removal enhanced neointimal hyperplasia following endovascular injury. Transplantation of subcutaneous fat from a normal mouse to surround the injured artery markedly attenuated neointimal formation. These observations suggest that PVAT may have a protective role in neointimal hyperplasia. Supporting this viewpoint, the atheroprotective effect of exogenous fat was not observed when subcutaneous fat was from obese mice. The explanation may be phenotypic changes in adipose tissue with the production of anti-inflammatory adiponectin decreasing and that of pro-inflammatory adipokines [interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), tumour necrosis factor-α (TNF-α) and plasminogen activator inhibitor type-1 (PAI-1)] increasing in subcutaneous fat-conditioned medium from obese mice compared with that from normal mice. The conditioned medium from the subcutaneous fat of normal mice attenuated VSMC proliferation produced by platelet-derived growth factor (PDGF)-BB. By contrast, the conditioned medium from the subcutaneous fat of obese mice increased VSMC proliferation, which was prevented by pretreatment with anti-TNF-α antibodies. Also, the conditioned medium of adiponectin-deficient subcutaneous fat stimulated VSMC proliferation. These findings reveal that TNF-α secreted from fat increased VSMC growth, and that adiponectin secreted from fat suppressed VSMC growth in response to PDGF-BB stimulation. Further experiments demonstrated that exogenous adiponectin suppressed PDGF-BB-induced VSMC proliferation via AMP-activated kinase pathway.

To analyse which molecule(s) contributes to the protection exerted by PVAT, Takaoka *et al*. focused on adiponectin. Adiponectin-deficient mice exhibited increased neointimal smooth muscle proliferation in response to endovascular injury, which was reversed by local perivascular delivery, but not systemic administration, of recombinant adiponectin. This indicates that adiponectin secreted from PVAT has a protective role in lesion formation in response to endovascular injury. Supporting this notion, the adiponectin expression

was down-regulated in the PVAT of obese mice, and endovascular injury-induced neointimal formation was enhanced in the artery of these mice.

In contrast to subcutaneous fat, transplantation of visceral fat to surround the injured artery exerted no protection (Takaoka *et al.*, 2009). The explanation for this difference may be the lower expression of adiponectin in visceral than subcutaneous fat in mice, similar to humans (Fain *et al.*, 2004). When considering the paracrine function of PVAT, it should be noted that PVAT heterogeneity exists in different vascular regions, different species and under different physiological and pathological conditions. In addition, the evidence for the protection by PVAT in the mouse femoral artery was mainly obtained using implanting subcutaneous fat rather than real PVAT, as the authors pointed out that it was impossible to obtain enough amount of PVAT for the transplantation to coat the artery of another mouse (Takaoka *et al.*, 2009).

In humans, plasma adiponectin levels in the cardiac vein were higher than those in the coronary artery, suggesting that adiponectin is produced locally in the coronary circulation (Date *et al.*, 2006). Adiponectin was expressed in both periaortic and pericoronary adipose tissue and negatively correlated with age and atherosclerosis (Spiroglou *et al.*, 2010). Adiponectin expression in epicardial adipose tissue surrounding the right coronary artery was decreased in metabolic syndrome (Teijeira-Fernandez *et al.*, 2011) and CAD patients (Iacobellis *et al.*, 2005; Karastergiou *et al.*, 2010). These studies suggest that low adiponectin level in local PVAT contributes to human atherosclerosis, as does systemic hypoadiponectinaemia.

TNF-α

Since it was reported in 1993 that adipose tissue expressed both the substantial TNF-α gene and one of candidate molecules inducing insulin resistance (Hotamisligil *et al.*, 1993), TNF-α has been recognized as an important adipokine. Adipose TNF-α expression and plasma TNF-α level are increased in most animal models and human subjects with obesity and insulin resistance. In addition, this cytokine has a variety of functions in inflammation and atherosclerosis (Matsuzawa, 2005).

After identifying adiponectin for the protection of PVAT against neointimal formation of the injured mouse femoral artery (Takaoka *et al.*, 2009), the same group proposed that TNF-α is involved in rapid phenotypic changes in PVAT following endovascular injury (Takaoka *et al.*, 2010). In addition to the model of wire injury in the mouse femoral artery, they demonstrated in another model (rat iliac artery balloon injury) that endovascular injury significantly up-regulated pro-inflammatory adipokines (MCP-1, TNF-α, IL-6 and PAI-1) and down-regulated the anti-inflammatory adipokine adiponectin within PVAT. TNF-α knockout attenuated up-regulation of pro-inflammatory adipokine expression in PVAT, with reduced neointimal hyperplasia after vascular injury. Local delivery of TNF-α to the perivascular area enhanced pro-inflammatory adipokine expression, and this was associated with augmented neointimal hyperplasia in TNF-α-deficient mice. Conditioned medium from a coculture of 3T3-L1 adipocytes and RAW264 macrophages stimulated VSMC proliferation. An anti-TNF-α neutralizing antibody in the coculture abrogated the stimulating effect of the conditioned medium. These findings suggest that endovascular injury-induced phenotypic changes in PVAT may play a role in the pathogenesis of neointimal hyperplasia after angioplasty, in which TNF-α is a major mediator. One possible mechanism for PVAT phenotypic alterations is that endothelial injury brings about infiltration of inflammatory cells (such as macrophages) into the vascular wall from the luminal side. Inflammatory cell-derived TNF-α may trigger adipocyte dysfunction and then lead to a vicious cycle between adipocytes and macrophages, which may aggravate inflammatory changes in the PVAT, demonstrated as up-regulation of the pro-inflammatory adipokines (MCP-1, TNF-α, IL-6 and PAI-1) and down-regulation of the anti-inflammatory adipokine (adiponectin) (Takaoka *et al.*, 2010).

However, the pathological effect of PVAT in this study (Takaoka *et al.*, 2010) is in contradiction with their previous finding that removal of PVAT enhanced neointimal hyperplasia after endovascular injury, indicating that PVAT may protect against neointimal formation after angioplasty (Takaoka *et al.*, 2009). Thus, the *in vivo* effect of PVAT needs to be studied further in different vascular regions, different species and under different physiological and pathological conditions.

In addition, TNF-α was found to be highly expressed in the balloon-injured rat aorta, but not in normal blood vessels (Jovinge *et al.*, 1997). Blockade of TNF-α activity by soluble TNF-α receptor suppressed coronary artery neointimal formation after cardiac transplantation in rabbits (Clausell *et al.*, 1994). TNF-α stimulated VSMC proliferation and migration *in vitro* (Wang *et al.*, 2001a; Kim *et al.*, 2007). The secretion of TNF-α in epicardial fat adjacent to the proximal coronary artery and abdominal fat was higher in CAD patients (Cheng *et al.*, 2008). These observations suggest PVAT-derived TNF-α may be involved in the VSMC growth and migration during vascular injury. To exactly identify the major molecule(s) for the role of PVAT in the proliferative vascular disease, the function of local PVAT from normal and diseased arteries should be compared using PVAT-conditioned medium and various specific blockers. For these studies, adipose-specific over- and under-expression would be more useful than systemic over- and under-expression.

Adipose-derived factors potentially involved in regulating VSMC growth by PVAT

Renin–angiotensin system (RAS)

RAS is very important in cardiovascular physiopathology and pharmacology (Fyhrquist and Saijonmaa, 2008; Abassi *et al.*, 2009). Adipose RAS and its functional importance have attracted closer attention (Kershaw and Flier, 2004; Thatcher *et al.*, 2009). Angiotensinogen was the first RAS component demonstrated in adipocytes and is abundantly expressed in adipose tissue. Adipose tissue, rather than the liver, may serve as a primary source of angiotensinogen in the foetus (Gomez *et al.*, 1988). Adipocyte-specific overexpression of angiotensinogen elevated both circulating angiotensinogen and blood pressure, indicating that adipocyte-derived angiotensinogen contributes to the systemic RAS and to the regulation of

Table 1

Gene microarrays of renin-angiotensin system in rat perivascular and subcutaneous adipose tissue

Gene	Mean expression value			Differential expression score		
	PVAT	MAT	SAT	PVAT versus MAT	PVAT versus SAT	MAT versus SAT
Renin	1	–1	3	0.0	0.0	0.0
Atp6ap2 (renin/prorenin receptor)	2046	4024	5339	–39.6**	–55.6**	–7.0
Igf2r (renin/prorenin clearance receptor)	2332	1829	2004	5.9	0.2	–2.1
Angiotensinogen	2217	3967	3122	–25.1**	–6.9	4.9
ACE	114	68	351	3.9	–55.5**	–101.5**
ACE2	89	37	52	8.1	5.3	–0.6
Chymase	1260	1016	4880	1.9	–82.5**	–99.1**
AT_{1A}	1224	1556	4259	–3.1	–79.8**	–62.7**
AT_{1B}	11	6	7	0.0	0.0	0.0
AT_2	–4	–4	–2	0.0	0.0	0.0
Mas (previous AT_3)	31	13	67	0.0	–1.4	–14.9*
IRAP (previous AT_4)	0	–6	1	0.0	0.0	–0.1
Aminopeptidase A	20	8	8	0.0	0.0	0.0
Aminopeptidase N	454	328	1967	6.4	–71.2**	–90.8**

Sprague–Dawley rats are used at the age of 27–28 weeks. $n = 8$.
*$P < 0.05$, 13 < differential expression score < –13; **$P < 0.01$, 20 < differential expression score < –20.
MAT, mesenteric adipose tissue; SAT, subcutaneous adipose tissue of inguinal region.

arterial blood pressure (Massiera *et al.*, 2001). In our study of rat gene microarrays, we analysed the expression of all RAS genes in adipose tissue (Table 1). The results showed that almost all RAS genes were expressed, except renin, AT_2 and insulin-regulated aminopeptidase (IRAP) receptor. The gene expression of the AT_{1A} receptor was high, whereas that of other genes encoding angiotensin receptors (AT_{1B}, AT_2, Mas and IRAP) was low in adipose tissue, indicating that effects of Ang II on adipose tissue are mediated mainly by AT_{1A} under physiological conditions. The two types of renin/prorenin receptors were both highly expressed in adipose tissue, indicating that adipose has the ability for renin/prorenin uptake and clearance, and that circulating renin/prorenin may affect adipose through the specific renin/prorenin receptor-mediated intracellular signalling and renin/prorenin activation (Nguyen, 2007).

In the rat aorta, the majority of angiotensinogen mRNA expression was localized in PVAT, and this protein was released into the medium from incubated adipose tissue with levels increasing over a 2 h period (Cassis *et al.*, 1988a; Cassis *et al.*, 1988b). Expression of angiotensinogen mRNA has been detected in human epicardial adipose tissue close to the course of the right coronary artery and significantly increased by acute surgery stress (Roubicek *et al.*, 2008). In addition to angiotensinogen mRNA, angiotensin-converting enzyme (ACE), ACE2 and chymase mRNAs were expressed in PVAT surrounding rat aortae and mesenteric arteries (Galvez-Prieto *et al.*, 2008) (Table 1). Renin/prorenin uptake was present, as indicated by high expression of two renin/prorenin receptors in perivascular fat (Table 1). Thus, PVAT contains the components necessary for the synthesis of angiotensin (Ang) II and Ang 1–7, two major effectors in the RAS. Ang II and Ang 1–7 were detected in rat periaortic and/or perimesenteric adipose tissue (Galvez-Prieto *et al.*, 2008; Lee *et al.*, 2009; Lu *et al.*, 2010). The expression of the specific renin/prorenin receptor and angiotensinogen was higher in mesenteric than periaortic adipose tissue (Table 1), supporting the finding that Ang II content was higher in mesenteric fat (Galvez-Prieto *et al.*, 2008). PVAT-derived Ang II is critically involved in the potentiation of vasoconstriction to perivascular neuronal stimulation in rat mesenteric arteries (Lu *et al.*, 2010), whereas PVAT-derived Ang 1–7 acted as a relaxing factor in rat aortas (Lee *et al.*, 2009; Lu *et al.*, 2011), indicating that the local RAS in perivascular fat plays a paracrine role in the regulation of vascular tone.

Although there has been no direct evidence regarding the effect of perivascular adipose RAS on VSMC growth and migration, it is reasonable to deduce that this local RAS may have this effect under physiological and/or pathological conditions. Indeed, Ang II is a powerful growth factor, stimulating VSMC growth (hypertrophy and hyperplasia) and migration *in vitro* and *in vivo* following activation of AT_1 (Kim and Iwao, 2000). ACE inhibitors and AT_1 antagonists reverse or reduce vascular remodelling in response to hypertension and vascular injury (Heeneman *et al.*, 2007). Of note, AT_2, expressed in the developing vascular system and re-expressed in adult VSMCs in response to vascular injury, has opposite functions, inhibiting proliferation (Nakajima *et al.*, 1995) and migration (Chassagne *et al.*, 2002). In addition, exogenous Ang 1–7 inhibits VSMC proliferation in a balloon-catheter injury model (Strawn *et al.*, 1999). It inhibits Ang II-induced VSMC proliferation and migration partly through negative modulation of Ang II-induced ERK1/2 activity (Zhang *et al.*, 2010) and down-regulation of AT_1 receptors in VSMCs (Clark

et al., 2001). The systemic infusion of Ang II induces local PVAT inflammation which may participate in hypertensive vascular remodelling (Guzik *et al.*, 2007) and abdominal aortic aneurysm formation (Police *et al.*, 2009). Nevertheless, further studies are needed to directly test whether local RAS in PVAT is involved in vascular diseases, as well as to identify which components play a role. Using adipose-specific over- or under-expression of individual RAS components would provide definitive evidence in this regard.

Reactive oxygen species (ROS)

The production of ROS increased selectively in adipose tissue of obese mice, and this was accompanied by an augmented expression of NADPH oxidase and a decreased expression of antioxidative enzymes (Furukawa *et al.*, 2004). PVAT-derived ROS was involved in the modulation of vascular contraction (Gao *et al.*, 2006; Gao *et al.*, 2007) and promoted endothelial dysfunction in diet-induced obese mice (Ketonen *et al.*, 2010). In New Zealand obese mice, a model for metabolic syndrome, PVAT of mesenteric resistance arteries showed increased superoxide anion production and NADPH oxidase activity, accompanied by hypertrophic vascular remodelling and loss of PVAT anti-contractile properties (Marchesi *et al.*, 2009). Epicardial adipose tissue suffered greater oxidative stress than subcutaneous adipose tissue in patients with cardiovascular disease (Salgado-Somoza *et al.*, 2010). ROS are important signalling molecules or second messengers that regulate proliferation, hypertrophy and migration of VSMCs (Taniyama and Griendling, 2003). It is generally believed that low concentrations of ROS induce cell growth, whereas high concentrations result in cell death (Taniyama and Griendling, 2003). Several factors have been shown to induce ROS-dependent migration of VSMCs, including PDGF (Weber *et al.*, 2004), thrombin (Wang *et al.*, 2004), vascular endothelial growth factor (Wang *et al.*, 2001b), MCP-1 (Lo *et al.*, 2005) and insulin-like growth factor 1 (Meng *et al.*, 2008). Actually, H_2O_2 treatment is sufficient to induce VSMC migration (Kim *et al.*, 2004).

Complement component 3 (C3)

PVAT-derived C3 stimulated adventitial fibroblast migration and differentiation. Indeed, C3 was increased in PVAT and associated with adventitial thickening and myofibroblast clustering around PVAT in deoxycorticosterone acetate-salt-hypertensive rats (Ruan *et al.*, 2010). C3 is involved in the synthetic phenotype and exaggerated growth of VSMCs from spontaneously hypertensive rats (Lin *et al.*, 2004), is essential for C-reactive protein-mediated exaggeration of neointimal formation in injured mouse carotid arteries (Hage *et al.*, 2010), is increased in atherosclerotic lesions and contributes to the development of the latter by stimulation of several cytokines *in vivo* (Buono *et al.*, 2002).

NO

NO can be released by adipocytes and endothelial cells of vasa vasorum in adipose tissue (Dashwood *et al.*, 2007; Malinowski *et al.*, 2008). NO production was increased in mesenteric PVAT, improving relaxation to acetylcholine and sodium nitroprusside, during early diet-induced obesity (Gil-Ortega *et al.*, 2010). Of the three NOS isoenzymes, eNOS immunostaining was the most abundant in PVAT of the saphenous vein, and eNOS activity was comparable in PVAT and the underlying vein, suggesting that PVAT-derived NO plays a role in the improved saphenous vein graft patency in patients undergoing coronary artery bypass surgery (Dashwood *et al.*, 2007). NO induces opposite effects on cell migration and proliferation depending on the different phenotypes of VSMCs. It inhibited the proliferation and migration of relatively dedifferentiated VSMCs, including subcultured cells or cells isolated from newborn rats (Garg and Hassid, 1989; Tanner *et al.*, 2000). For differentiated VSMCs isolated from the adult rat aorta, NO potentiated the effect of growth factors on cell proliferation and induced migration (Brown *et al.*, 1999; Chang *et al.*, 2002). In addition, different NOS isoforms produce converse effects on VSMC growth and migration. Gene konockout studies indicated that eNOS suppresses vascular injury-induced neointima formation (Moroi *et al.*, 1998), whereas iNOS promotes this neointima formation (Chyu *et al.*, 1999). iNOS expression in adipose tissue is up-regulated in obesity, a low-grade chronic inflammation (Perreault and Marette, 2001). It remains to be answered whether this iNOS up-regulation occurs in the inflammatory PVAT and thus contributes to the obesity-associated vascular disease.

Hydrogen sulphide (H_2S)

Rat aortic PVAT expressed cystathionase, a key enzyme for H_2S generation, and released H_2S. The H_2S production decreased in an age-dependent manner (Fang *et al.*, 2009). PVAT-derived H_2S has been identified as a relaxing factor (Fang *et al.*, 2009). Atorvastatin increases net H_2S production in PVAT, but not media, by inhibiting its mitochondrial oxidation and results in the augmentation of anti-contractile effect of PVAT (Wojcicka *et al.*, 2011). H_2S inhibited VSMC proliferation and induced VSMC apoptosis in vitro (Du *et al.*, 2004). Endogenous H_2S may protect against atherosclerosis (Qiao *et al.*, 2010) and neointima hyperplasia following endovascular injury (Meng *et al.*, 2007). Enhanced cell proliferation has been detected in the aortic media from cystathionase knockout mice (Yang *et al.*, 2010).

Other factors

PVAT expresses some other known adipokines, most of which have been verified for their secretion from PVAT. Given their roles in the regulation of VSMC growth, they may be potentially PVAT-derived growth factors or inhibitors. These factors include MCP-1 (Egashira *et al.*, 2002; Chatterjee *et al.*, 2009; Takaoka *et al.*, 2010), IL-6 (Yao *et al.*, 2007; Langheim *et al.*, 2010; Takaoka *et al.*, 2010), IL-8 (Yue *et al.*, 1994; Henrichot *et al.*, 2005; Chatterjee *et al.*, 2009), leptin (Li *et al.*, 2005; Zeidan *et al.*, 2005; Cheng *et al.*, 2008; Ketonen *et al.*, 2010), resistin (Calabro *et al.*, 2004; Langheim *et al.*, 2010), PAI-1 (Chen *et al.*, 2006; Takaoka *et al.*, 2009; Karastergiou *et al.*, 2010), adrenomedullin (Kano *et al.*, 1996; Iwasaki *et al.*, 1998; Shibasaki *et al.*, 2010), heparin-binding epidermal growth factor-like growth factor (HB-EGF) (Igura *et al.*, 1996; Matsumoto *et al.*, 2002), IL-10 (Selzman *et al.*, 1998; Eiras *et al.*, 2010), IL-1β (Dinarello, 1996; Lohmann *et al.*, 2009) and macrophage migration inhibitory factor (MIF) (Pan *et al.*, 2004; Karastergiou *et al.*, 2010).

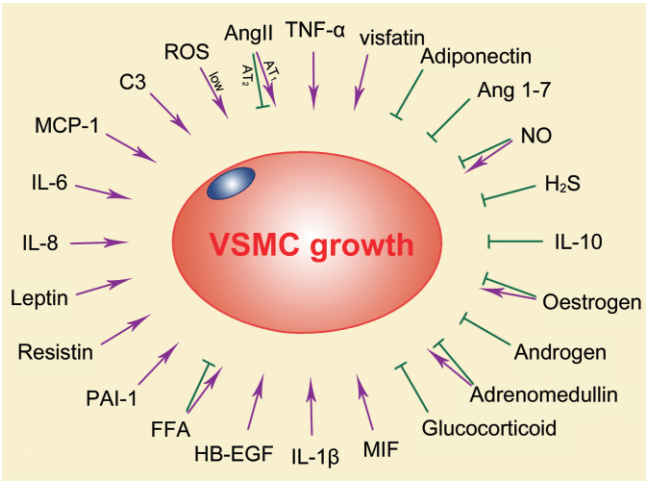

Figure 3
Effects of adipose-derived factors on VSMC growth.

Epicardial adipose tissue has a greater capacity for FFA release than the perirenal and popliteal adipose tissue (Marchington *et al.*, 1989). Saturated fatty acids are higher and unsaturated fatty acids are lower in epicardial adipose tissue near the proximal right coronary artery than subcutaneous adipose tissue (Pezeshkian *et al.*, 2009). Actually, the nature of FFAs released by different regional perivascular fats is still unclear, and the compositions of PVAT-derived FFAs should be defined when verifying their local effect, since different FFAs exert distinct effects on VSMC growth (Rao *et al.*, 1995; Pakala and Benedict, 1999; Schauer and Reusch, 2009; Lamers *et al.*, 2011).

Glucocorticoids inhibit VSMC proliferation and migration *in vitro* (Longenecker *et al.*, 1984; Pross *et al.*, 2002). They prevent restenosis after angioplasty in patients (Liu *et al.*, 2004). Adipocytes highly express 11-β-hydroxysteroid dehydrogenase 1 (11β-HSD1) that converts inactive glucocorticoid precursors (cortisone) to active glucocorticoids (cortisol) in humans (Seckl and Walker, 2001). 11β-HSD1 mRNA is increased in epicardial adipose tissue near the proximal tract of the right coronary artery in CAD patients (Silaghi *et al.*, 2007). However, there is no information concerning the function of PVAT-derived glucocorticoids.

Adipose tissue produces sex hormones from precursors. It is speculated that 10–20% of the body production of sex steroids indeed are formed in adipose tissue (Frayn *et al.*, 2003). Oestrogen suppresses (Sullivan *et al.*, 1995; Jiang *et al.*, 2010) or enhances (Keyes *et al.*, 1996; Ling *et al.*, 2004) VSMC growth, depending on cellular phenotype (Song *et al.*, 1998). Androgen reduces neointimal formation in rabbit carotid balloon injury model (Ii *et al.*, 2009) and exerts an anti-proliferative, pro-apoptotic and anti-migrative effect on cultured VSMCs (Furutama *et al.*, 1998; Bowles *et al.*, 2007).

Overall, among the abovementioned factors, mainly visfatin, adiponectin and TNF-α appear to participate in the paracrine regulation by PVAT of VSMC growth and migration (Figure 3). PVAT-derived Ang II, Ang 1–7, ROS, C3, NO and H_2S have a paracrine action on VSMC contraction, endothelial function or fibroblast function; however, their paracrine action on VSMC growth and migration remains to be directly verified. More work is needed to conclude as to the importance of the remaining factors (MCP-1, IL-6, IL-8, leptin, resistin, PAI-1, adrenomedullin, HB-EGF, IL-10, IL-1β, MIF, FFAs, glucocorticoids and sex hormones) as regards a contribution to the paracrine role of PVAT in the regulation of VSMC growth and migration.

PVAT and vascular disease

The epicardial fat volume is well correlated with cardiovascular risk factors (Rosito *et al.*, 2008). Epicardial fat, but not intrathoracic fat, is associated with coronary artery calcification, whereas intrathoracic fat, but not epicardial fat, is associated with abdominal aortic calcification (Rosito *et al.*, 2008). CAD is negatively related to coronary flow hyperaemia, and among the fat depots, epicardial fat is the only independent predictor of hyperaemic myocardial perfusion (Bucci *et al.*, 2011). These studies support the hypothesis that PVAT exerts a paracrine effect in vascular disease. A correlation also exists between coronary PVAT and atherosclerosis, as the amount of local pericoronary fat appears to be related to the plaque burden in the underlying coronary artery segment (Mahabadi *et al.*, 2010; Wang *et al.*, 2010b). The relationship between PVAT and atherosclerosis has been reviewed elsewhere (Verhagen and Visseren, 2011).

PVAT may participate in restenosis after angioplasty. Indeed, inflammatory cells increased in PVAT of the porcine coronary artery after angioplasty (Okamoto *et al.*, 2001). Similar results were observed in PVAT of mouse femoral artery and rat iliac artery after endovascular injury, with up-regulation of pro-inflammatory and down-regulation of anti-inflammatory adipokines (Takaoka *et al.*, 2010). These phenotypic changes in PVAT were enhanced by diet-induced obesity in mice and were involved in neointimal formation following endovascular injury (Takaoka *et al.*, 2009; Takaoka *et al.*, 2010).

PVAT surrounding saphenous vein may reduce vein graft stenosis, improving early (1.5 years) and long-term (8.5 years) graft patency in patients with coronary artery bypass grafting (Souza *et al.*, 2001; Souza *et al.*, 2006). These suggest that PVAT prevents intimal hyperplasia and atherosclerosis after saphenous vein grafting (Souza *et al.*, 2006). Several mechanisms may be considered for the beneficial effects of PVAT-intact saphenous vein grafts, such as anti-spasm, anti-platelet, anti-proliferative and mechanical actions as well as the minimal injury on the vascular wall (Souza *et al.*, 2001; Souza *et al.*, 2006). PVAT-derived NO may be one of the potential molecules for the protection of PVAT in saphenous vein grafts (Dashwood *et al.*, 2007).

Besides local adipocyte dysfunction, the increase of inflammatory cells in PVAT is involved in vascular disease. Macrophages and T cells in PVAT are increased in obesity (Police *et al.*, 2009; Takaoka *et al.*, 2009), vascular injury (Takaoka *et al.*, 2010), aging (Fei *et al.*, 2010), acute systemic infection (Madjid *et al.*, 2007) and atherosclerosis (Henrichot *et al.*, 2005; Lohmann *et al.*, 2009). T cells play a role in the genesis of Ang II-induced hypertension and vascular dysfunction (Guzik *et al.*, 2007). Systemic infusion of Ang II induces local PVAT inflammation and obvious T-cell infiltration,

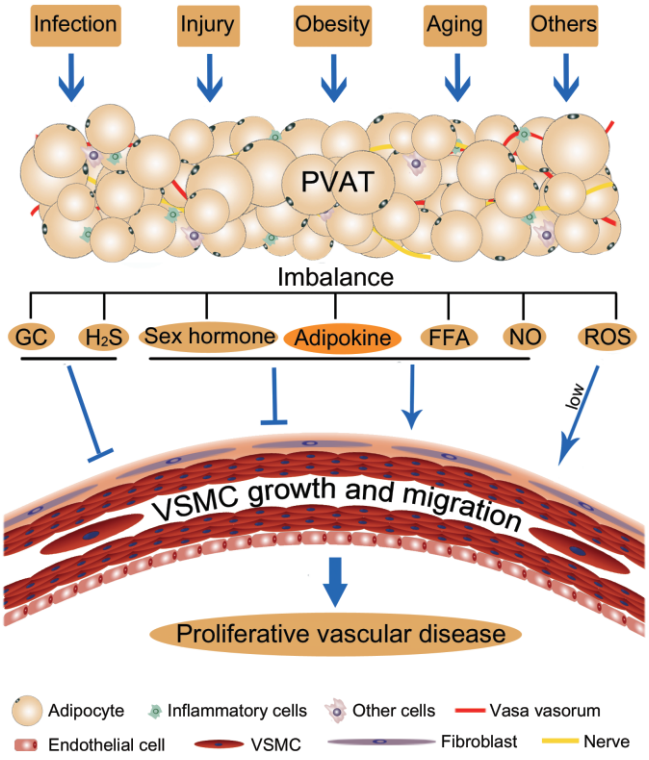

Figure 4

Schematic diagram for perivascular fat dysfunction and vascular disease. Obesity, aging, vascular injury, infection and other factors may affect PVAT, causing adipocyte abnormality and inflammatory cell infiltration, leading to imbalance of PVAT-secreted growth factors and inhibitors, tending towards VSMC growth and migration and finally resulting in development of proliferative vascular disease. GC, glucocorticoid.

which may participate in hypertensive vascular remodelling (Guzik *et al.*, 2007). Adipose tissue secretes chemokines (Henrichot *et al.*, 2005) and has a loose structure to recruit and accommodate inflammatory cells, which can further alter adipose tissue function (Weisberg *et al.*, 2003). The resulting adipocyte abnormality and inflammatory cell infiltration may induce imbalance of PVAT-derived factors, for example, secreting more growth factors and less inhibitors, leading to VSMC growth and migration, and finally resulting in development of proliferative vascular disease (Figure 4). One of the key features of cell migration is the capacity of the cells to breakdown extracellular matrix components by matrix metalloproteinases (MMPs). In VSMCs, MMPs can be activated by TNF-α, Ang II, ROS, leptin, resistin, IL-6 and MIF (Moon *et al.*, 2004; Kong *et al.*, 2005; Li *et al.*, 2005; Wang *et al.*, 2005; Chen *et al.*, 2007; Hu *et al.*, 2009; Ding *et al.*, 2011), which can be released by PVAT, especially during inflammation. These further support the proposed mechanisms for involvement of PVAT dysfunction in vascular disease (Figure 4).

Perspective

Current research on perivascular fat opens a new field in vascular biology. Both the intimal endothelium and the adventitial fat regulate contraction and growth of medial VSMCs. The crosstalk between cells in the blood vessel wall is vital for normal vascular function. Imbalance of PVAT-derived factors may play a role in proliferative vascular diseases such as atherosclerosis, restenosis and hypertension. To identify key factors altered in distinct sites of PVAT and involved in stimulation of proliferation may help to develop new therapies for the prevention and treatment of vascular disease. Using *in vivo* models of cell-specific gene over- and under-expression, systemic gene over- and under-expression, and local pharmacological interventions may provide definitive evidence for drug target identification. Antibodies, recombinant proteins and chemicals may be used locally, for example during angioplasty or vein grafting. Preservation of PVAT may be as important as preservation of endothelium for a successful vascular intervention.

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Conflict of interest

None.

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