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Review

PVAT: an important guardian of the cardiovascular system

Xiuying Liang*, Yan Qi*, Fan Dai, Jingya Gu and Wenjuan Yao
School of Pharmacy, Nantong University, Nantong, China
*These authors contributed equally to this work

Summary. Perivascular adipose tissue (PVAT) had long been considered to serve only structural, vessel-supporting purposes, but today PVAT is recognized to be an endocrine organ with important physiological and pathological effects. The expansion of PVAT in vascular homeostasis and vascular disease has attracted much interest. PVAT has been shown to release a wide spectrum of molecules, such as PVAT-derived relaxing factors (PVATRFs) and PVAT-derived contracting factors (PVATCFs). PVAT dysfunction may lead to obesity, atherosclerosis, and other cardiovascular diseases. This review describes recent advances in our understanding of PVAT's important effects on the cardiovascular system.

Key words: Perivascular adipose tissue, Adipocytes, PVAT-derived relaxing factor, PVAT-derived contracting factor

Introduction

Perivascular adipose tissue (PVAT) is fat tissue with endocrine and paracrine functions surrounding the blood vessels, such as the small mesenteric and femoral arteries or large abdominal aorta (AA), and plays wide-ranging physiological roles beyond merely being a supporting scaffold (Fernández-Alfonso et al., 2017). Adipocytes are the main cellular component of PVAT and have been reported to differentiate from PVAT stem cells towards smooth muscle cells (SMC) regulated by mi378a-3p (Gu et al., 2019). There are also reports showing that adipogenic progenitors *in vivo* are adventitial fibroblasts and not mural cells (Guimarães-Camboa and Evans, 2017). Furthermore, PVASCs (stromal cells in the PVAT) have the capacity for brown adipogenic differentiation, which is decreased during aging via the loss of peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) (Pan et al., 2019). However, the exact source of adipocytes in PVAT and whether that source is the same as for other fat depots is still unclear. It has been reported that PVAT has anti-contractile effects through the adrenergic system with the release and uptake of norepinephrine (NE) (Sena et al., 2017). In addition, PVAT adipocytes have the novel ability to store NE in a VMAT (vesicular monoamine transporter)-dependent manner, indicating that there is an independent adrenergic system within PVAT (Ahmad et al., 2019). Therefore, PVAT is likely to be an important participant in blood pressure regulation. PVAT is a unique adipose tissue depot that contributes to vascular homeostasis, and there are many studies showing that PVAT dysfunction may induce several pathophysiological situations, such as obesity, diabetes, or hypertension due to changes in its amount or in the expression pattern of vasoactive factors (Greenstein et al., 2009). In this review, we provide an update on the effects of PVAT on the cardiovascular system.

Offprint requests to: Wenjuan Yao, Department of Pharmacology, School of Pharmacy Nantong University, 19 QiXiu Road, Nantong 2260001, Jiangsu, China. e-mail: yaowenjuan0430@aliyun.com
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Adipose cell types in PVAT

PVAT surrounding the vasculature consists of many types of cells but is dominated by perivascular

adipocytes. PVAT includes the following adipose cell types: brown adipose cells containing multiple lipid droplets and densely packed mitochondria that express thermogenic genes (like mitochondrial uncoupling protein-1, UCP-1); white adipose cells (like mesenteric PVAT) with large lipid droplets and relatively low expression levels of UCP-1; and beige or bright (brown-in-white) adipose cells (like coronary PVAT) that express UCP-1 and other characteristic markers, such as CD137, Tbx1, and Cited-1 (Harms and Seale, 2013; Cheng et al., 2018). Brown adipose tissue (BAT) in the human body is mainly found in the supraclavicular, neck, perirenal, and mediastinal regions (Hildebrand et al., 2018). Brown adipose cell differentiation is regulated by several factors such as peroxisome proliferator-activated receptor gamma (PPARγ), PGC-1α, orexin, and bone morphogenic factor 7 (BMP7) (Tseng et al., 2008; Hondares et al., 2011; Cohen et al., 2014). White adipose tissue (WAT) is mainly located in subcutaneous regions and surrounding internal organs and secretes hormones and cytokines such as leptin, adiponectin, tumor-necrosis factor α (TNFα), and interleukin-6 (IL-6) (Chen et al., 2017). White adipocyte differentiation is regulated by transcription factors including CCAAT/enhancer-binding-proteins C/EBPβ, C/EBPδ, C/EBPα, and PPARγ (Hildebrand et al., 2018). The capacity of WAT for browning is controlled by the expression of PR domain containing 16 (PRDM16), which is critical for phenotypic maintenance of classical BAT (Seale et al., 2011). Table 1 shows a comparison between the different adipose cells discussed in this review.

Several reports have indicated that thoracic periaortic adipose tissue is morphologically similar to classical BAT, but not WAT (Fitzgibbons et al., 2011; Chang et al., 2012). However, there are other reports suggesting that the adipose tissue surrounding the abdominal aorta or mesentery is similar to WAT (Gálvez-Prieto et al., 2008; Police et al., 2009; Omar et al., 2014). In contrast, coronary PVAT is more like beige adipose tissue, and the expression levels of brown adipocyte-related genes are apparently different from those of classical BAT (Wu et al., 2012; Lian and Gollasch, 2016). The transcriptional profile of A-T-PVAT (anterior thoracic aorta PVAT) is distinctly different from those of LL-T-PVAT (left lateral thoracic aorta PVAT) and RL-T-PVAT (right lateral thoracic aorta PVAT). The differentially expressed genes are associated with the following biological processes: aorta development, angiogenesis, connective tissue development, and adipogenesis (Ye et al., 2019). Therefore, PVAT from different anatomical locations has different structures and compositions of adipocytes. Table 2 shows the different adipose cells in different PVATs.

Vasoactive factors released by PVAT

PVAT is now considered to be a metabolically active organ that mediates communication with vascular cells in both an autocrine and paracrine fashion via the production of a variety of molecules. PVAT secretes a wide spectrum of candidate molecules, including perivascular factors (like PVAT-derived relaxing factor, PVATRFs; and PVAT-derived contracting factor, PVATCFs), adipokines (like adipokines and angiotensins), inflammatory cytokines, and possibly miRNAs. In this review, we focus on PVATRFs and PVATCFs (Fig. 1).

PVAT-derived relaxing factors, PVATRFs

Intact aortic rings surrounded with fat release a substance, most likely a protein, to rapidly relax pre-contracted arteries. This has been called "adventitium-derived relaxing factor" (ADRF), now also known as PVATRF (Löhn et al., 2002). Palmitic acid methyl ester (PAME) was previously considered to be the PVATRF, since it causes vasorelaxation by opening voltage-dependent K^+ channels on smooth muscle cells. Diminished PAME release and its vasorelaxing activity and increased release of angiotensin II in PVAT suggest a novel role for PVAT in the pathogenesis of hypertension (Lee et al., 2011). Therefore, the effects of antihypertensive drugs such as losartan may be attributed partly to their enhancement of PAME-induced vasorelaxation. In addition, adipocyte-derived nitric oxide (NO) plays a central role in anticontractile activity when rodent PVAT is stimulated by noradrenaline (Bussey et al., 2018). Noradrenaline and adipocyte-

Table 1. Comparison between different adipose cell types.

	White adipose cell	Brown adipose cell	Beige adipose cell
Morphological characteristics	Large lipid droplets	Multiple lipid droplets Densely packed mitochondria	Multiple lipid droplets Densely packed mitochondria
Distribution	Subcutaneous regions Internal organs	Supraclavicular, neck, perirenal and mediastinal region	WAT in a "browning" process
Differentiation regulation	C/EBPβ, C/EBPδ, C/EBPα, PPARγ	PPARγ, PGC-lα, orexin, BMP7	PRDM16
Markers	Low expression levels of UCP-1	UCP-1	UCP-1, CD137, Tbx1, Cited-1

CCAAT/enhancer-binding-proteins (C/EBPβ, C/EBPδ, C/EBPα), peroxisome proliferator-activated receptor gamma (PPARγ), uncoupling protein-1 (UCP-1), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), bone morphogenic factor 7 (BMP7), positive regulatory domain containing 16 (PRDM16), and white adipose tissue (WAT).

located β3-adrenoceptor stimulation lead to activation of Gαs signaling pathways dependent upon Kv7 channel function and thus promote the release of adipocyte-derived NO (Bussey et al., 2018). Another report has suggested that the control of vascular tone triggered by PVAT mainly involves Mas receptor and AT2 receptor activation with consequent PI3k/Akt pathway and NO synthase activation (Nóbrega et al., 2019). It has been demonstrated that PVAT activates the Mas receptor and its downstream PI3k/Akt pathway and nNOS (neuronal nitric oxide synthase) activation, thus leading to an increase in the production of NO and H_2O_2. PVAT can also activate the AT2 receptor and eNOS (endothelium nitric oxide synthase) isoform in a less important way during the process (Zhu et al., 2013; Zhao et al., 2014; Nóbrega et al., 2019). Furthermore, the volatile gaseous mediator H_2S, which mediates the anti-contractile effect by opening KCNQ channels in VSMCs, has been suggested to be a primary candidate for PVATRF. However, this view is still controversial because there is no anti-contractile effect of H_2S in mice (Cheng et al., 2018; Szijártó et al., 2018). It has been reported that PVAT generates prostaglandin I2 (PGI2) and improves high-fat diet (HFD)-impaired endothelial function of ApoE -/- mouse carotid arteries (Chang et al., 2012). The renin-angiotensin system (RAS) from PVAT gives rise to the production of the heptapeptide Ang1-7 by angiotensin-converting enzyme 2 (ACE2), which serves as a vasodilator (Hass et al., 2014). Inhibition of both MAO (monoamine oxidase) and SSAO (semicarbazide sensitive amine oxidase) increases the potency of NE at mesenteric arteries with PVAT, though inhibition of MAO-A/B or SSAO individually dose not alter contraction by NE. Meanwhile, inhibition of the norepinephrine transporter (NET) with nisoxetine also reduces PVAT's anti-contractile effect on NE. Therefore, PVAT's uptake and metabolism of NE may contribute to its anti-contractile effect (Ayala-Lopez et al., 2017). Despite several candidates being proposed, the real identity of PVATRF and its precise mechanism of vasodilatory effect are unknown.

PVAT also secretes other critical vasoregulatory candidate subtypes, such as adiponectin, leptin, TNF-α, and IL-6 (Cheng et al., 2018). Adiponectin exerts many other vasculoprotective and angiogenic properties (Shibata et al., 2004). Angiogenic repair of ischemic hind limbs is impaired in adiponectin-KO mice

Table 2. Fat cell types in different PVAT.

PVAT of different blood vessels	Adipose cell types	References
Thoracic aorta	BAT	Baker et al., 2006
Periaortic	BAT	Trujillo et al., 2005
Abdominal aorta	WAT	Watts et al., 2013; Song et al., 2014
Mesenteric	WAT	Ayala-Lopez et al., 2014
Coronary	Beige	Cui et al., 2014; Owen et al., 2014

perivascular adipose tissue (PVAT), brown adipose tissue (BAT), and white adipose tissue (WAT).

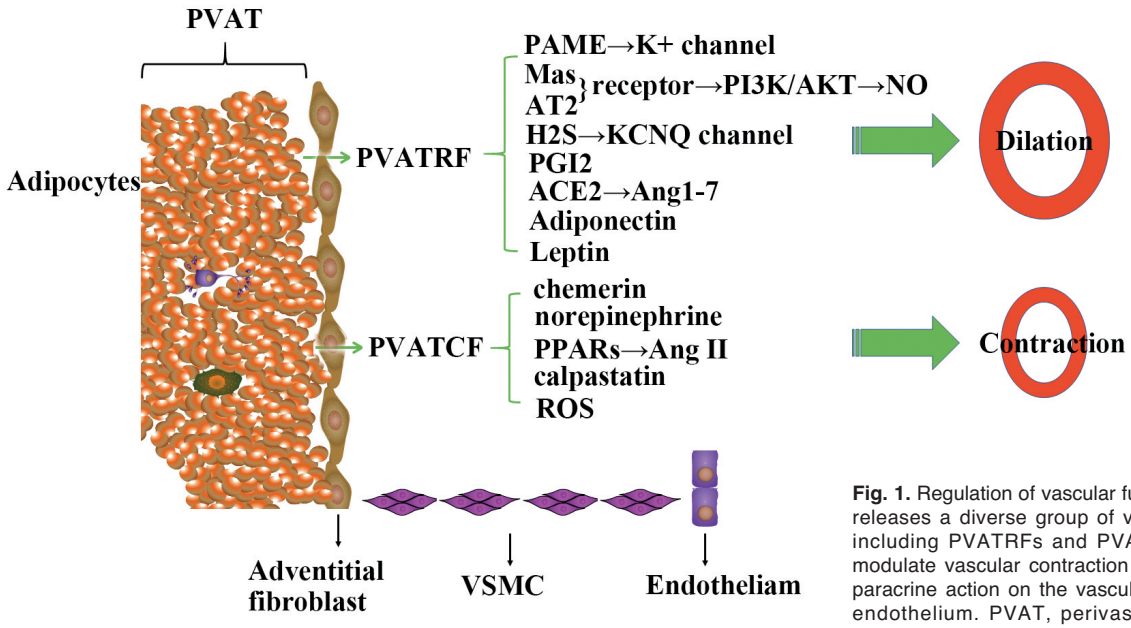

Fig. 1. Regulation of vascular function by PVAT. PVAT releases a diverse group of vasoactive substances, including PVATRFs and PVATCFs. These factors modulate vascular contraction and dilation through a paracrine action on the vascular smooth muscle and endothelium. PVAT, perivascular adipose tissue; VSMC, vascular smooth muscle cell; PVATRF, PVAT-derived relaxing factor; PVATCF, PVAT-derived contracting factor; PAME, palmitic acid methyl ester; ACE2, angiotensin converting enzyme 2; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species.

compared with wild-type (WT) mice, which can be reversed by adenovirus-mediated supplementation with adiponectin. Adiponectin functions to stimulate angiogenesis in response to ischemic stress by promoting AMP-activated kinase signaling. Leptin is the product of the obese gene (ob) and regulates feeding behavior. It has been reported that leptin promotes vascular inflammation and VSMC proliferation (Nakamura et al., 2014). Leptin is structurally similar to the helical cytokine family that includes IL-2 and acts on multiple types of immune cells to promote the release of inflammatory cytokines and transformation of T cells toward a T_H1 cell phenotype (Nakamura et al., 2014). Leptin and adiponectin secreted exclusively by adipose tissue were originally considered as a PVATRF (Trujillo and Scherer, 2005; Baker et al., 2006). However, leptin and adiponectin induce direct vasodilation that depends on an intact and functional endothelium.

PVAT-derived contracting factor, PVATCFs

Apart from PVATRFs, PVAT also produces PVATCFs to modulate vasoconstriction, such as chemerin (Watts et al., 2013; Song et al., 2014), norepinephrine (NE) (Ayala-Lopez et al., 2014; Cui et al., 2014), calpastatin (Guan et al., 2014; Owen et al., 2014), and ROS (Gao et al., 2006). The primary chemerin receptor agonist ChemR23 causes contraction in isolated rat thoracic aorta when nitric oxide synthase is inhibited, endothelial cells are mechanically removed, or a tone is placed on the arteries. The novel ChemR23 antagonist CCX832 inhibites phenylephrine- and PGF2α-induced contraction (+PVAT), suggesting that endogenous chemerin contributes to contraction (Watts et al., 2013; Song et al., 2014). PVAT components that are independent of sympathetic nerves can release NE in a tyramine-sensitive manner to result in arterial contraction, suggesting the local control of arterial function by PVAT catecholamines (Ayala-Lopez et al., 2014; Cui et al., 2014). Augmented contractile effects of obese coronary PVAT are related to calpastatin upregulation in the PVAT proteome. Calpastatin (10 μM) peptide incubation in lean coronary arteries without PVAT increases contractions to levels similar to those observed in the presence of PVAT (Guan et al., 2014; Owen et al., 2014). Furthermore, PVAT enhances the arterial contractile response to perivascular nerve stimulation through the production of superoxide mediated by NAD(P)H oxidase, and this enhancement involves the activation of tyrosine kinase and the MAPK/ERK pathway. Inhibitors of NAD(P)H oxidase and cyclooxygenase exert a greater inhibition on EFS (electrical field stimulation)-induced contraction in PVAT (+) mesenteric arteries than in PVAT (-) mesenteric arteries. Inhibitors of tyrosine kinase (tyrphostin A25) and MAPK/ERK (U0126) attenuate EFS-induced contractions in PVAT (+) mesenteric arteries (Gao et al., 2006; Wang et al., 2015).

Local RAS from PVAT contributes to many diseases, such as obesity/insulin resistance, and hypertension (Phillips et al., 1993). Renin transcription in PVAT can be positively regulated by PPARs (Kupiers et al., 2008). In addition, PPARγ is required for functional PVAT development, and PPARγ deficiency enhances atherosclerosis and vascular and systemic inflammation (Xiong et al., 2018). Deletion of PPARγ in brown adipocytes impaires the development of PVAT and increases the basal expression of inflammatory genes and macrophage infiltration in PVAT. Atherosclerotic lesions are significantly increased in mice with impaired PVAT development by crossing BA-PPARγ-KO mice with ApoE KO mice (Xiong et al., 2018). Ang II has been reported to stimulate adipocytes differentiation through angiotensin type 1 receptor (AT1R) (Matsushita et al., 2006). Blockade of the effects of endogenous angiotensin II by incubation with valsartan alone inhibites adipogenesis, whereas PD123319 alone promotes adipogenesis, suggesting distinct roles for AT1 and AT2 receptors in adipocyte differentiation (Matsushita et al., 2006). In addition, increased Ang II levels may induce reactive oxygen species (ROS) during obesity in systemic and related adipose tissue (Okada et al., 2010; Zhang et al., 2015). Treatment of 3T3-L1 and primary adipocytes with reactive oxygen species (hydrogen peroxide) causes a significant decrease in the expression and secretion of angiotensinogen (AGT), which is suppressed by treatment with the antioxidant N-acetyl cysteine. Furthermore, Ang II mediates the PVAT-associated increase of contractile response to perivascular neuronal excitation (Lu et al., 2010; Xu et al., 2017). In rat mesenteric arteries, treatment of the vessels with an ACE inhibitor (enalaprilat) or angiotensin II type 1 receptor antagonist (candesartan) reduces the PVAT-mediated potentiation of EFS-induced contraction. Exogenously applied angiotensin II enhances EFS-induced contraction in arteries without PVAT, but not in arteries with intact PVAT (Lu et al., 2010). It has been reported that local Bmal1 in PVAT regulates Agt expression and the ensuing increase in Ang II, which acts on smooth muscle cells (SMCs) in the vessel walls to regulate vasoactivity and blood pressure in a circadian fashion during the resting phase (Chang et al., 2018). However, the precise function of RAS in PVAT is still controversial.

PVAT and cardiovascular diseases

Recent studies have revealed that PVAT dysfunction induces pathophysiological states such as obesity, atherosclerosis, or hypertension (Figs. 2, 3). Mice deficient in the tumor CDKN2A/p16 ^{INK4a} develop more epicardial adipose tissue in response to the adipogenic PPARγ agonist rosiglitazone (Wouters et al., 2017). Mice transplanted with p16 INK4a -deficient bone marrow have more epicardial adipose tissue compared to controls when fed a high-fat diet. In humans, epicardial adipose tissue from obese humans displays an increased expression of stem cell antigens compared to lean

controls. An HFD induces the downregulation of *rictor* expression in thoracic periaortic PVAT and thus increases the release of proinflammatory proteins (TNF-α, IL-6, and MCP-1) (Bhattacharya et al., 2013). Also, the anti-contractile effects of PVAT are reduced in small arteries of patients with metabolic syndrome or obesity (Greenstein et al., 2009). In contrast, diet-induced weight loss reverses obesity-induced PVAT damage through a mechanism involving reduced inflammation and increased NO synthase activity within PVAT (Bussey et al., 2016). These data reveal inflammation and NO synthase, particularly endothelial NO synthase, as potential targets for the treatment of PVAT dysfunction associated with obesity and metabolic syndrome. PVAT also triggers early-stage vascular remodeling in the context of obesity-associated inflammation by an EV-miR-221-3p-mediated mechanism (Li et al., 2019). In addition, some research indicates that antiretroviral therapy (ART) and a high-calorie diet (HCD), separately and combined, alter both

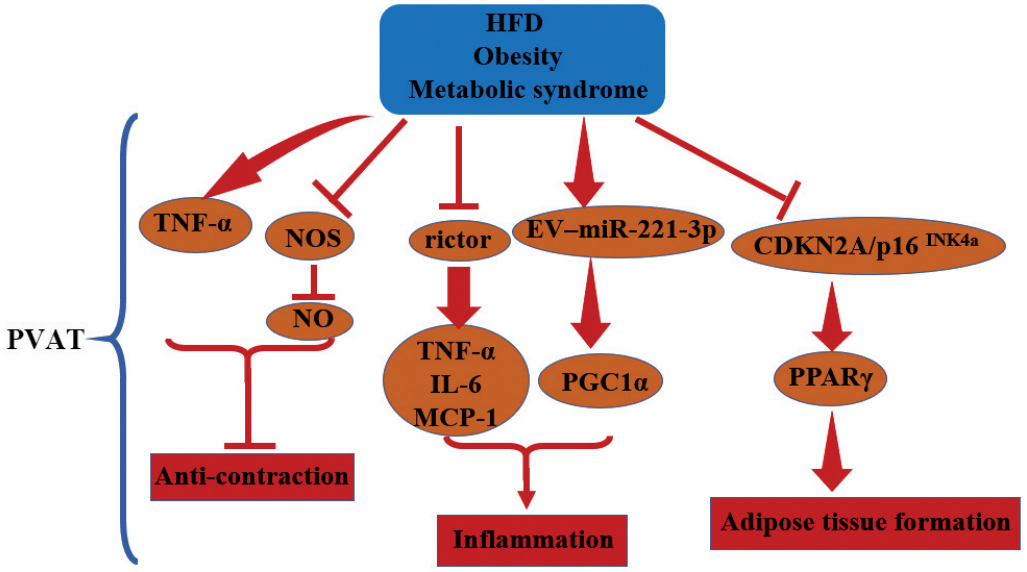

Fig. 2. PVAT dysfunction in obesity and metabolic syndrome. Obesity promotes the secretion of inflammatory factors and PPARs, which leads to PVAT dysfunction characterized by decreased anti-contractile effects and increased inflammation and fat formation. HFD, high fat diet; NO, nitric oxide; NOS, NO synthetase; TNF-α, tumor necrosis factor α; IL, Interleukin; MCP, monocyte chemo- attractant protein; PPARγ, peroxisome proliferator-activated receptor γ; PGC1α, PPARγ coactivator 1α.

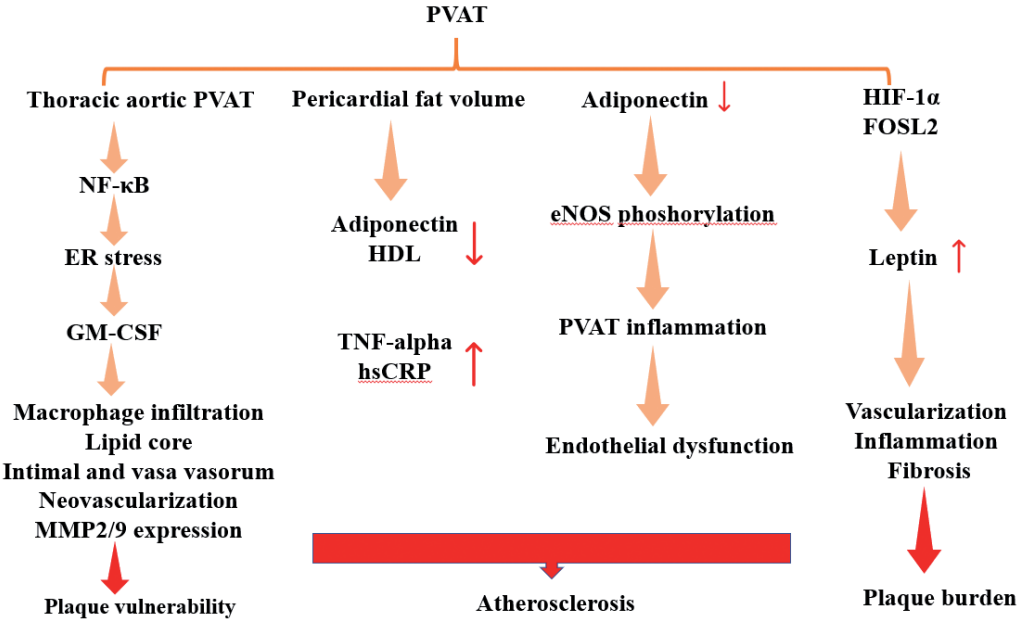

Fig. 3. PVAT dysfunction leads to atherosclerosis. PVAT participates in the atherosclerosis process and plaque stabilization through a variety of mechanisms. NF-κB, nuclear factor kappa-B; ER, endoplasmic reticulum; GM-CSF, granulocyte macrophage colony-stimulating factor; MMP, matrix metalloproteinase; HDL, high-density lipoprotein; hs-CRP, hypersensitive C-reactive protein; HIF-1α, hypoxia-inducible factor-1; FOSL2, Fos-like antigen 2.

the tunica media and adventitia of the aortic wall in rats (Nel et al., 2017). Changes seen with ART in the rat model suggest that aortic wall thickness and PVAT adipocyte morphology alterations should be considered by clinicians treating obese individuals receiving ART. Overall, PVAT dysfunction contributes to the pathogenesis of obesity and metabolic syndrome.

PVAT has also been reported to be associated with atherosclerosis and plaque stabilization. It has been reported that an elevated PVAT volume is strongly associated with coronary atherosclerosis (Greif et al., 2009). Additional experimental data have shown that transplanted PVAT promotes plaque vulnerability in a setting of HFD, which can be ameliorated by 4-PBA (4-phenyl butyric acid) at least in part dependent on decreased GM-CSF (granulocyte macrophage colony-stimulating factor) released locally by transplanted PVAT (Ying et al., 2018). This finding demonstrates a direct relationship between endoplasmic reticulum (ER) stress in PVAT and plaque destabilization and implies that GM-CSF secretion by PVAT is a mediator of this pathological process. Genetic deletion of Ang II type 1a (AT 1a) receptor in PVAT can attenuate aortic aneurysm formation, macrophage infiltration, and gelatinolytic activity in apolipoprotein E-deficient (ApoE –/–) mice. This study indicates a previously unrecognized effect of the AT 1a receptor in PVAT in the pathogenesis of abdominal aortic aneurysms (Sakaue et al., 2017). Moreover, adiponectin normalizes endothelial cell function by a mechanism that involves increased endothelial NO synthase (eNOS) phosphorylation and decreased PVAT inflammation. Detailed characterization of the adiponectin signaling pathway in the vasculature and perivascular fat is likely to provide novel approaches to the management of atherosclerosis and metabolic disease (Sena et al., 2017). Furthermore, suppression of the pro-inflammatory phenotype of PVAT might contribute, at least partially, to atherogenesis inhibition and cardiovascular protection (Salim et al., 2017). In humans, a higher degree of local tissue hypoxia and upregulation of leptin expression in the PVAT, along with increased vascularization, inflammation, and fibrosis, may contribute to increased atherosclerotic plaque burden in the coronary arteries compared to the inferior mesenteric arteries (IMA) in patients (Drosos et al., 2016).

Conclusions

By increasing our knowledge of the structure and function of PVAT, we may better understand the role of PVAT in cardiovascular maintenance and disease. However, despite a much better understanding of PVAT, there are still many unanswered questions. It is apparent that PVAT surrounding different blood vessels may play different physiological/pathophysiological roles. Many researchers have proposed that PVAT should be considered as a specialized organ with the ability of differentially modulating the vascular function at each anatomical location (Greenstein et al., 2009). Additionally, the crosstalk between PVAT and other adipose tissues in other depots should also be studied in the future. Moreover, whether the signaling pathway from external to internal (PVAT-SMC-EC) mediates vascular homeostasis and diseases remains to be confirmed. In contrast, the signaling from inside to outside (EC-SMC-PVAT) may participate in PVAT functional maintenance or impairment. In many previous studies, 3T3-L1 adipocytes served as a classic model for PVAT adipocytes *in vitro*. However, recent research indicates that 3T3-L1 adipocytes are not an adequate model of perivascular adipocytes for studying NE handling (Ismail et al., 2017). Therefore, PVAT-specific animal models or tools are currently lacking.

It is undeniable that PVAT is a unique tissue depot that contributes to vascular homeostasis while it also has the potential to participate in certain pathological conditions. However, more efforts are needed to clarify the differences between the mechanisms of endothelium-induced and PVAT-induced relaxation and the crosstalk between PVAT and other adipose tissues in other depots. Furthermore, more studies are required to determine the detailed transcriptome-proteome of PVAT isolated from humans, as well as to characterize PVAT-derived vasoactive factors together with their precise mechanisms. Meanwhile, researchers need to establish appropriate PVAT-specific genetic animal models and tools. Regardless, targeting PVAT is a promising therapeutic strategy for treating and preventing vascular diseases.

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