REVIEW ARTICLE

Perivascular Adipose Tissue: the Sixth Man of the Cardiovascular System

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Abstract

Perivascular adipose tissue (PVAT) refers to the local aggregate of adipose tissue surrounding the vascular tree, exhibiting phenotypes from white to brown and beige adipocytes. Although PVAT has long been regarded as simply a structural unit providing mechanical support to vasculature, it is now gaining reputation as an integral endocrine/paracrine component, in addition to the well-established modulator endothelium, in regulating vascular tone. Since the discovery of anti-contractile effect of PVAT in 1991, the use of multiple rodent models of reduced amounts of PVAT has revealed its regulatory role in vascular remodeling and cardiovascular implications, including atherosclerosis. PVAT does not only release PVAT-derived relaxing factors (PVRFs) to activate multiple subsets of endothelial and vascular smooth muscle potassium channels and anti-inflammatory signals in the vasculature, but it does also provide an interface for neuron-adipocyte interactions in the vascular wall to regulate arterial vascular tone. In this review, we outline our current understanding towards PVAT and attempt to provide hints about future studies that can sharpen the therapeutic potential of PVAT against cardiovascular diseases and their complications.

Keywords Adipose tissue · Perivascular relaxing factor · PVRF · KCNQ-type K_V7 channels · Beige adipocytes · Exercise · Perivascular nerves

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Key points • A functionally unique and specialized adipose tissue aggregate, termed perivascular adipose tissue (PVAT), encapsulates most of the blood vessels within the vascular tree.
• PVAT demonstrates phenotypic and functional heterogeneity, sandwiched between brown adipose tissue and white adipose tissue. PVAT microenvironment demonstrates heterogeneity, consisting of numerous cell types and molecules.
• Various animal models, either after specialized pretreatment protocol, with modified PVAT content or of human disease background are commonly used to verify the pathophysiological roles of PVAT in cardiovascular events.
• PVAT derives a wide spectrum of candidate molecules, including perivascular contracting/relaxing factors, adipocytokines including adipokines and inflammatory cytokines, and possibly miRNAs to regulate vascular tone, vascular inflammation, and vascular remodeling.

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Abbreviations

ACE	Angiotensin-converting enzyme
ADicerKO	miRNA-processing enzyme Dicer
AdipoR	Adiponectin receptor
ADRF	Adipocyte-derived relaxing factor
Akt	Protein kinase B
AMPK	AMP-activated protein kinase
Ang1-7	Angiotensin 1-7
Ang II	Angiotensin II
AT1	Angiotensin II type 1
AT_{1a}	Angiotensin II type 1a
AT2	Angiotensin II type 2
ATP	Adenosine triphosphate
aP2	Acid-binding protein
ApoE	Apolipoprotein E
APN	Aminopeptidase
AR	Adrenoceptor
ASP	Acylation stimulation protein
BAT	Brown adipose tissue
BCA	β-cyano-L-alanine
BK_{ca}	Large conductance calcium-activated potassium
cAMP	Cyclic adenosine monophosphate
CAST	Calpastatin
cGMP	Cyclic guanosine monophosphate
CGRP	Calcitonin gene-related peptide
ChemR23	Chemerin receptor 23
CIDEA	Cell death-inducing DNA fragmentation factor-α-like effector A
cir-miRNA	Circulating miRNA
COX	Cyclooxygenase
CPT_1B	Carnitine palmitoyltransferase $_1$B
CSE/CTH	Cystathionine gamma lyase
CVD	Cardiovascular disease
CXC	Cysteine-X-cysteine
CYP 450	Cytochrome P450
DRF	Dorsal root ganglia
DIO	Diet-induced obesity
EDHF	Endothelium-dependent hyperpolarizing factor
EET	Epoxyeicosatrienoic acid
EFS	Electric field stimulation
eNOS	Endothelial nitric oxide synthase
ERK	Extracellular signal-regulated kinase
FAT-ATTAC	Fat apoptosis through targeted activation of caspase 8
FGF2	Fibroblast growth factor 2
FKBP	FK506 binding protein
GC	Guanylyl cyclase
H_2O_2	Hydrogen peroxide
HFD	High-fat diet
HF/HS	High-fat high-sucrose
H_2S	Hydrogen sulfide
HSV	Herpes simplex virus-1

IGF-1	Insulin-like growth factor-1
IKK	I kappa B kinase
IL-1	Interleukin 1
IL-1β	Interleukin 1β
IL-6	Interleukin 6
IL-6R	IL-6 receptor
IL-8	Interleukin 8
IP	Prostacyclin receptor
K_{ATP}	ATP-sensitive potassium channels
KCNQ	Potassium voltage-gated channel subfamily Q
K_{dr}	Delayed-rectifier potassium channel
K_v	Voltage-gated potassium channel
LDL	Low-density lipoprotein
LDLR	LDL receptor
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemotactic protein-1
miRNA	microRNA
MSC	Mesenchymal stem cell
NA	Noradrenaline
NAD(P)H	Nicotinamide adenine dinucleotide phosphate
NANC	Non-adrenergic non-cholinergic
NE	Norepinephrine
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NO	Nitric oxide
NMN	Nicotinamide mononucleotide
NPY	Neuropeptide Y
NZO	New Zealand obese
O_2^-	Superoxide
OCT3	Organic cation transporter 3
PAI-1	Plasminogen activator inhibitor-1
PAME	Palmitic acid methyl ester
PAT	Periaortic adipose tissue
PG	Prostaglandin
PGD_2	Prostaglandin D2
PGE2	Prostaglandin E2
$PGF_{2\alpha}$	Prostaglandin F2α
PGI_2	Prostaglandin I2
PGP	Protein gene product
PPG	DL-propargylglycine
PVAT	Perivascular adipose tissue
PVCF	Perivascular contraction factor
PVRF	PVAT-derived relaxing factor
RAS	Renin-angiotensin system
ROS	Reactive oxygen species
SCID	Severe combined immunodeficiency
SFRP5	Secreted frizzled-related protein 5
SHR	Spontaneously hypertensive rat
SMC	Smooth muscle cell
SMPG KO	Smooth muscle cell-selective PPARγ knockout
SNA	Sympathetic nerve activity
SNS	Sympathetic nervous system

SP	Sensory neurotransmitter
TNF-α	Tumor necrosis factor-α
TRPV1	Transient receptor potential cation channel sub-family V member 1
TXA$_2$	Thromboxane A2
UCP-1	Uncoupling protein 1
VEGF	Vascular endothelial growth factor
VEGFA	Vascular endothelial growth factor A
VSMC	Vascular smooth muscle cell
WAT	White adipose tissue
WKY	Wistar-Kyoto rat
Wnt5a	Wingless-related MMTV integration site 5A
WT	Wild-type
XE991	4-Pyridinylmethyl-9(10H)-anthracenone

Introduction

Perivascular Adipose Tissue

The vascular bed is a composite system enriched by various types of blood vessels, each specialized to adapt to the metabolic and hemodynamic requirements of a specific organ. Most of the blood vessels within the vascular tree are surrounded by a functionally unique and specialized adipose tissue aggregate differing from general fat depots, termed perivascular adipose tissue (PVAT) [1]. Staying in close proximity to the tunica adventitia of large-, medium-, and small-diametered arteries, PVAT is a crucial paracrine/endocrine organ for cardiometabolic regulation [2]. Except the cerebral vasculature, PVAT widely distributes around large arteries and veins, small and resistance vessels, skeletal muscle microvessels, and within organs, including kidney in the renal sinus around both renal artery and vein [3]. During tumor expansion, adipose tissue around tumor vasculature plays a pivotal role in secreting angiogenic factors [(as fibroblast growth factor 2 (FGF2) and vascular endothelial growth factor A (VEGFA)), adipokines (as adiponectin and leptin), and cytokines (as interleukin-6 (IL-6))], all of which contribute to a pro-angiogenic microenvironment [4], though whether those adipose tissue belongs to PVAT remains to be discussed. Under the stress of exogenous risk (as smoking and obesity), PVAT might act as a negative modulator in the progression of type 2 diabetes and cardiovascular diseases (CVDs), including hypertension, atherosclerosis, and coronary heart diseases [5, 6]. In a genome-wide expression investigation of in vitro differentiated adipocytes derived from paired subcutaneous and coronary perivascular adipose tissues, which were isolated from patients, a signature of 307 differentially expressed genes mainly for the regulation of vascular morphology, angiogenesis, blood clotting, and inflammation was identified [7].

PVAT Heterogeneity

Depending on the regions in the vascular bed where PVAT is located, PVAT displays phenotypic and functional heterogeneity [3, 8]. PVAT majorly exhibits several defining features of brown adipose tissue (BAT), including the presence of multiple lipid droplets, high mitochondrial content, and expression of thermogenic genes (like *Ucp-1*) [9]. However, certain PVAT (like mesenteric PVAT) has been characterized as white adipose tissue (WAT)-like, due to the presence of relatively large lipid droplets, low mitochondrial content, and the relatively low expression levels of uncoupling protein 1 (UCP-1) [10]. In contrast, certain PVAT (like coronary PVAT) is more beige adipose tissue-like since the expression levels of brown adipocyte-related genes, including cell death-inducing DNA fragmentation factor-α-like effector A (CIDEA), UCP-1, and carnitine palmitoyltransferase $_1$B (CPT$_1$B), are apparently different from those of typical BAT in studies using fat deposits collected from 129SVE mice and human [11, 12]. Hence, with features that could hardly fit into the predefined fat tissue, it is reasonable to categorize PVAT as the fourth type of adipose tissue [9, 13].

PVAT Microenvironment

PVAT plays active paracrine/endocrine roles in regulating arterial vascular tone of enclosed blood vessels and its microenvironment consists of various cell types and molecules (Fig. 1). Above tunica adventitia, adipocytes within PVAT (either brown-like, white-like, or beige-like) are responsible for the production of many biologically active molecules, with possible autocrine and/or paracrine effects, modulating vascular function and cardiometabolic complications [13, 14]. In brief, these molecules can be categorized into putative vasoactivators, including both perivascular contraction factors (PVCFs) and perivascular relaxing factors (PVRFs) [15, 16], adipokines like adiponectin, leptin, and resistin, cytokines such as tumor necrosis factor-α (TNF-α) and interleukins, and certain gaseous molecules like nitric oxide (NO) and hydrogen sulfide (H$_2$S) [17, 18]. Through secretion of cytokines during the pathogenesis of atherosclerosis, PVAT was confirmed, in both rodents and human atherosclerotic aortae [19], to promote the recruitment and infiltration of immune cells, including macrophages and T cells, which in terms produce additional chemokines initiating a feed-forward loop for inflammatory response [19, 20]. Furthermore, resident multipotent stem cells within PVAT, named mesenchymal stem cells (MSCs), show multiple differentiation capacity towards adipocytes, endothelial cells, smooth muscle cells (SMCs) and osteoblasts for repair and replacement [21–23]. For instance, isolated human perivascular stem cells were able to undergo in vitro osteogenic differentiation and form bone in vivo after intramuscular implantation in male severe

Fig. 1 PVAT microenvironment. PVAT surrounding vasculature consists of many types of cells, dominated by perivascular adipocytes (either brown-like or white-like depending on the region of vascular bed). PVAT around atherosclerotic aortae recruits immune cells, such as macrophages and T cells, responsible for the production of pro-inflammatory cytokines during atherogenesis. Resident stem cells within PVAT show multipotency towards adipocytes, endothelial cells, and smooth muscle cells. PVAT also provides an interface for neuron-adipocyte interactions and releases microvesicles and/or exosomes bearing miRNAs to either enter circulation or modulate adipose tissue inflammation
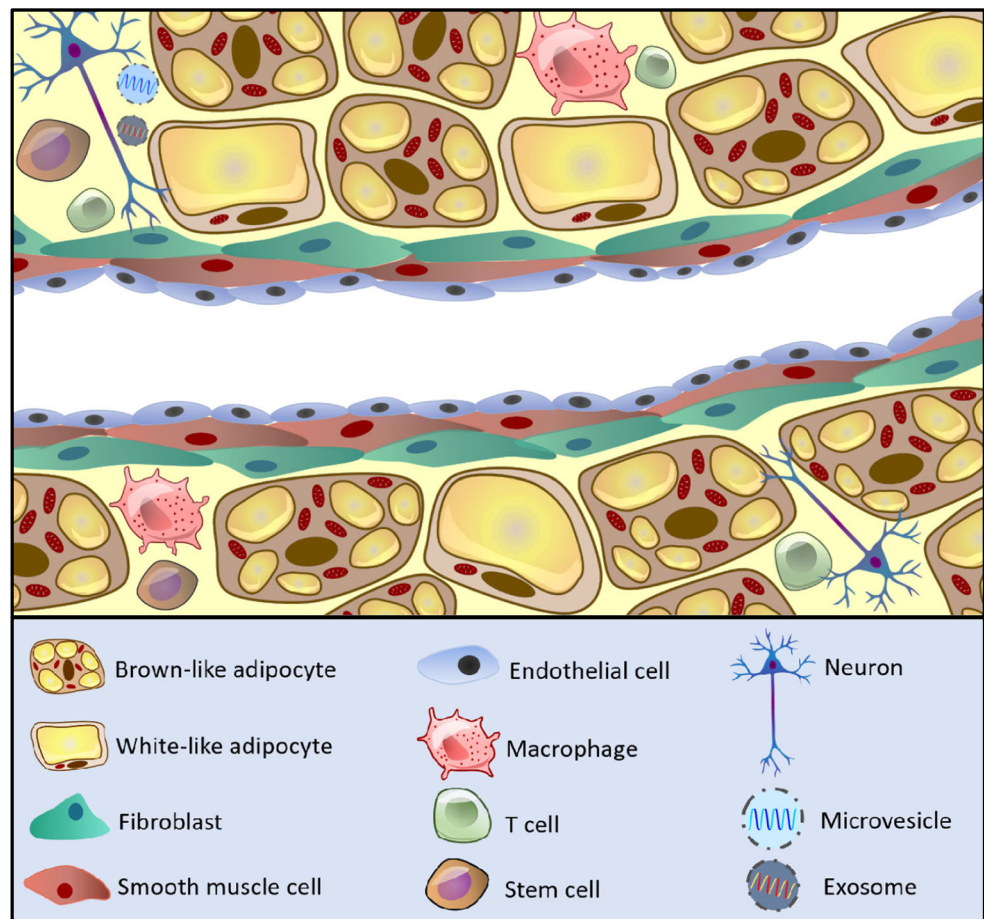
combined immunodeficiency (SCID) mice [24]. PVAT also provides an interface for interactions between perivascular adipocytes and autonomic perivascular nerve [25]. In brief, perivascular adipocytes express different receptors, including adrenoceptors (ARs), receptors for neuropeptide Y (NPY), and purinoceptors, sensing neurotransmitters secreted by adjacent sympathetic nerve [25, 26]. Recently, studies also provide hints that PVAT might be a potential source of microRNAs (miRNAs) by releasing microvesicles or exosomes, which may either enter circulation or transfer into nearby adipocytes or macrophages for endocrine functions and inflammatory communications [27–29].

Experimental Model

In addition to its well-established roles in providing structural support to vasculature and in thermoregulation of intravascular temperature [30], PVAT plays significant roles in modulating the onset and progression of many CVDs, including atherosclerosis and hypertensive heart diseases [5, 31]. Various animal models, either after pretreatment protocol (Table 1), with modified PVAT content (Table 2), or of human disease background (Table 3), have been used to emphasize the

importance of PVAT in cardiovascular events. In this session, we attempt to summarize the major animal models in the context of vascular tone regulation, atherosclerosis, vascular remodeling, angiogenesis, and hypertensive disorders.

Pretreatment Protocol

Animals and/or their tissues might undergo acute or chronic procedure prior to experiments on vascular reactivity assay. Since the first-time discovery of anti-contractile effect of PVAT against norepinephrine on isolated rat aorta by Soltis and Cassis in 1991 [32], abundant acute ex vivo experiments investigating the effect of PVAT on vascular function have been carried out, using isolated blood vessels from rat, mouse, swine, rabbit, and human [32–37]. Depending on the nature of the study, the interaction between PVAT and different regions of vascular bed, like aorta, mesenteric artery, and coronary artery, were investigated [33, 38]. For instance, mouse small mesenteric arteries, together with PVAT, were isolated and mounted on a wire myograph to study how adiponectin activates large conductance calcium-activated potassium (BK_{ca}) channels or other pathways to induce anti-contractile responses ex vivo [39, 40].

Table 1 Experimental models with pretreatment protocols

Model	Description	Cardiovascular event(s)	Limitation	Ref.
Ex vivo experiments	• Isolated arteries from rat, mouse, swine, rabbit, or human • PVAT around aorta, mesenteric artery or coronary artery, etc.	• Vascular function	• Only acute experimental clues	[32–40]
Diet-induced obesity (DIO)	• Mice subjected to a high-fat diet, normally for 3 to 4 months (≥ 8 weeks)	• Vascular function • Vascular inflammation • Atherosclerosis	• Careful maintenance of animals	[41–43]
Exercise	• Rodents subjected to a chronic training on a treadmill designed for small animals	• Vascular function	• Highly redundant routine • Hard to draw a direct link between PVAT and improved outcome	[44, 45]
Transplantation	• Transplantation of exogenous PVAT to recipient animal • Replacement of PVAT with other fat depots including viscera and subcutaneous fat tissues	• Vascular remodeling • Atherosclerosis	• High standard surgical techniques required • Risk of infection • Careful maintenance of animals	[46–48]

In addition, chronic diet-induced obese animal model is generally used to provide pathophysiological mimetics to study the vasoregulatory roles of PVAT. Following the feeding of high-fat diet (HFD), normally lasting for 3 to 4 months, the mouse of diet-induced obesity (DIO) is sacrificed to evaluate the impact of PVAT on vascular function in the state of obesity [41, 42]. Using the C57BL/6J mouse model of DIO, Li and his group showed the role of endothelial nitric oxide synthase (eNOS) uncoupling in PVAT underlying obesity-related vascular dysfunction [43]. Recently, the significance of exercise is increasingly recognized, so research groups started to use chronic exercise animal model in their studies. After male Wistar rats were trained on a treadmill designated for small animals with individual lanes for about 8 weeks, the thoracic aortae with PVAT were isolated for vascular function assay [44, 45]. However, routine exercise results in broad effects in metabolic profile of trained animals that it may not be easy to draw a direct link between PVAT and the improved vascular function.

Transplantation is a common procedure in exploring the function of PVAT in atherogenesis and vascular remodeling. Normally, researchers might transplant PVAT from an artery to another or replace PVAT with viscera and subcutaneous adipose tissues [46, 47]. Takaoka et al. demonstrated that transplantation of subcutaneous fat from mice fed on normal chow could attenuate the enhanced neointima formation due to the removal of periadventitial adipose tissue in C57BL/6 mice fed with high-fat high-sucrose (HF/HS) diet [47]. Another study transplanted thoracic PVAT from donor mice to carotid arteries of recipient ApoE$^{-/-}$ mice to indicate the therapeutic potential of PVAT in promoting atherosclerotic plaque vulnerability [48].

Modified PVAT Content

In addition to the non-genomic experimental models, several transgenic rodent models with reduced or absent PVAT have been developed. The A-ZIP/F mouse is one of the currently

Table 2 Transgenic mouse models with reduced PVAT content

Model	Phenotype	Cardiovascular event(s)	Limitation	Ref.
A-ZIP/F	Complete ablation of WAT, reduced BAT and PVAT, fatty liver, diabetes, hyperlipidemia	• Not appropriate	• Too complex physiological phenotypes	[49, 50]
UCP-I-DT	Significant deletion of BAT, hyperphagia, diabetes, hypertriglyceridemia	• Unknown	• Incomplete deletion of BAT • Expression of 50% of *Ucp1* mRNA	[52, 53]
Transgenic skinny	Complete deletion of adipose tissue, elevated glucose metabolism, small liver	• Not suitable for vascular function and hypertension studies	• Overexpression of leptin • Lack of reports on PVAT status	[54, 55]
FAT-ATTAC	Phenotypes similar to A-ZIP/F mouse, remarkable ablation of WAT and BAT, reduced systemic inflammation, glucose intolerance	• Unknown	• Lack of reports on PVAT status	[56]
SMPG KO	Presence of BAT and WAT, absence of PVAT, hypotension during resting period of circadian cycle	• Not suitable for blood pressure-related topics	• Problem of hypotension	[30, 57]

Table 3 Rodent models with susceptibility to human diseases

Model	Phenotype	Cardiovascular event(s)	Limitation	Ref.
ApoE$^{-/-}$ mouse	Hypercholesterolemia, cardiac hypertrophy, poor vascular function, hypertension (aging)	• Atherosclerosis • Aneurysm	• Careful maintenance of animals • Specific diet needed	[58–61]
LDLr$^{-/-}$ mouse	Hypercholesterolemia, fatty streaks on the aorta, hypertension	• Atherosclerosis • Vascular function • Arterial stiffness	• Careful maintenance of animals • Specific diet required • Lower total plasma cholesterol levels than ApoE$^{-/-}$ mouse	[62–64]
SHR	Hypertension, vascular hypertrophy, cardiac hypertrophy	• Hypertension • Vascular function	• Larger size, more space required	[65]
NZO mice	Hypertension, diabetes, obesity	• Hypertension • Diabetes • PVAT malfunction	• Careful maintenance • Breeding	[66–69]

available models, in the absence of WAT, and with dramatically reduced PVAT and BAT during lifetime, resulting in complex pathological phenotypes including hypertension and diabetes mellitus [49]. In brief, the A-ZIP/F mouse expresses the dominant negative protein A-ZIP/F, under the control of an adipose-specific acid-binding protein (aP2) promoter [13, 49]. Although A-ZIP/F mouse exhibits altered vascular contractile activity, ex vivo co-incubation of its aorta with PVAT from wild-type (WT) mouse could not restore the impaired vascular function [50]. Furthermore, Takemori et al. showed that the expression level of angiotensin II (Ang II) type 1 receptors is higher than that of Ang II type 2 receptors [50]. These observations imply that the absence of PVAT may not be the cause of dysfunctional aortae, suggesting that A-ZIP/F mouse might not be suitable for understanding the interrelationship between PVAT and vascular tone regulation.

Several other mouse models with ablation of adipose tissue might be potentially suitable for PVAT study, including those of either inborn or inducible adipose deletion [51]. Generated in 1993, the UCP-I-DT transgenic mouse is considered one of the classical rodent models with complete ablation of BAT, with physiological phenotypes of hyperphagia, diabetes mellitus, and hypertriglyceridemia [52]. However, it was later reported that UCP-I-DT model still retains a significant number of brown adipocytes, expressing approximately 50% of *Ucp1* mRNA in the brown fat [53]. Another potential transgenic model for PVAT study is named transgenic skinny, characterized with complete ablation of adipose tissue and increased glucose metabolism [54]. However, this model is also accompanied by overexpression of leptin [54], meaning that it might complicate the study involving vascular tone and hypertension, in which leptin plays active regulatory roles [55].

In 2005, a relatively new mouse model named fat apoptosis through targeted activation of caspase 8 (FAT-ATTAC) was developed, through targeted utilizing of a caspase 8-FKBPv (Phe36Val mutant FK506 binding protein (FKBP)) fusion protein under the control of adipocyte-specific *Fa*bp4 promoter [56]. In short, through activation

of caspase 8 to induce fat apoptosis, this mouse exhibits phenotypes close to A-ZIP/F mouse showing reduced WAT and BAT content, reduced systemic inflammation, and glucose intolerance [56]. Despite the lowered fat content in these animals, there are still lacking reports on their PVAT status. In 2012, Chang and his colleagues developed a murine smooth muscle cell-selective PPARγ knockout (SMPG KO) model that is deficient in PPARγ in vascular smooth muscle cells (VSMCs), showing the presence of BAT and WAT depots but not PVAT [30]. However, SMPG KO mice were hypotensive during the resting period of circadian cycle [30, 57], which might not be suitable for examining the effect of PVAT on blood pressure regulation.

Susceptibility to Human Diseases

Rodents with susceptibility to human diseases remain the species of choice to address the important roles of PVAT in the progression of atherosclerosis, hypertension, and vascular remodeling. Both apolipoprotein E-knockout (ApoE$^{-/-}$) mouse and low-density lipoprotein (LDL) receptor-deficient mouse (LDLr$^{-/-}$) with significant higher LDL levels, are the standard models used in atherosclerosis research. These mice develop robust lesions to form atherosclerotic plaques on the Western or atherogenic diet [58]. Hence, many studies examined the role of PVAT using ApoE$^{-/-}$ mice. For example, transplantation of perivascular visceral adipose tissue from ApoE$^{-/-}$ donors to the right common carotid artery, a site that typically does not develop spontaneous atherosclerosis, of ApoE$^{-/-}$ recipients resulted in a more advanced atherosclerotic lesion with the evidence of atherothrombosis and endothelial dysfunction [59]. Another study reported that periaortic adipose tissue (PAT) activated the renin-angiotensin system (RAS) to accelerate chronic kidney disease-associated atherogenesis [60]. In addition, another study unraveled that PVAT contributes to aneurysm formation in an angiotensin II type 1a (AT$_{1a}$) receptor-dependent manner [61].

Few studies used LDLr$^{-/-}$ mouse, another well-recognized atherosclerotic model but with much lower total levels of plasma cholesterol than ApoE$^{-/-}$ mice under low fat chow diet [62]. However, the studies are not directly related to atherosclerosis that a recent report focused on how PAT-derived IL-6 contributes to arterial stiffness in LDLr$^{-/-}$ mice [63]. A most recent study shows the protective benefit of PAT in endothelial dysfunction and insulin-induced vasodilation of LDLr$^{-/-}$ mice [64]. Working on hypertensive rodent models provides useful clues about the contribution of PVAT to hypertension-related complications. Earlier studies examined the influence of hypertension on the anti-contractile effect of PVAT. Lu et al. suggested that changes in adipocyte composition of PVAT, but not its mass, might exacerbate vascular dysfunction in a spontaneously hypertensive rats (SHR) [65]. These studies were paralleled by studies using New Zealand obese (NZO) mice that exhibit PVAT malfunction on arterial tone regulation despite excess visceral adipose mass. The anti-contractile effects of PVAT were diminished in mesenteric arteries of NZO mice with age. Voltage-gated potassium channel subfamily Q (KCNQ) channel openers reduced arterial blood pressure in both models of hypertension independent of ganglionic blockade. Thus, our data suggest that KCNQ channels play a pivotal role in periadventitial vasoregulation of peripheral skeletal muscle and visceral arteries, and KCNQ channel opening may be an effective mechanism to improve impaired PVAT vasoregulation and associated hypertension [17, 66]. We also found that BK$_{Ca}$ channels and/or K$_v$1.5 channels in VSMCs are not the downstream mediators involved in the inhibitory effects of 4-pyridinylmethyl-9(10H)-anthracenone (XE991) (a KCNQ channel blocker) on PVAT-dependent arterial relaxation [67]. KCNQ1 channels are apparently not involved in the control of arterial tone by α1-adrenoceptor agonists and PVAT [68]. Based on preclinical and clinical data, KCNQ channels (in particular KCNQ2-5 channels (K$_v$7.2-5)) in VSMCs can now be viewed as potential novel drug targets for treatment of resistant hypertension, particularly in comorbid conditions such as obesity and metabolic syndrome [69]. Taken together, the available data allowed us to propose that dysfunctional PVAT contributes to cardiovascular risks [11, 17].

PVAT Candidates

Accumulating evidence suggests that PVAT is a significant autocrine/endocrine/paracrine organ surrounding the vasculature. By secreting a wide spectrum of candidate molecules, including perivascular factors (like PVRFs and PVCFs), adipokines (like adipokines and angiotensins), inflammatory cytokines, and possibly miRNAs, PVAT plays a pivotal role in regulating vascular tone, vascular inflammation, and vascular remodeling through targeting different cell types in

vasculature and even in the PVAT microenvironment including perivascular adipocyte itself (Fig. 2).

Perivascular Factors

Both PVAT and endothelium are now considered important paracrine tissues regulating the contractile state and homeostasis of underlying SMCs through the production of various substances [17]. PVAT adipocytes release bioactive signaling molecules, majorly categorized into PVRFs and PVCFs, to confer vasoregulatory effect [11]. Certain simple molecules (e.g., NO, H$_2$S), reactive oxygen species (e.g., hydrogen peroxide (H$_2$O$_2$)), and adipokines (e.g., adiponectin, angiotensin 1-7 (Ang1-7)) are notable PVRFs [6, 17], contributing to the anti-contractile effect of PVAT on vascular bed. In 1987, Palmer et al. remarkably discovered that endothelial cells produce NO, the critical endothelium-derived relaxing factor for proper vascular function [70]. Similar to endothelium, PVAT was shown to express eNOS to generate NO from L-arginine for the regulation of vascular tone [71], through NO-mediated upregulation of cyclic guanosine monophosphate (cGMP) levels and subsequent activation of protein kinase G [72, 73]. Importantly, knockout of protein kinase G accounted for the loss of anti-contractile effect of PVAT on the mouse mesenteric arteries [73].

Among PVRFs, adipocyte-derived relaxing factor (ADRF) is a transferable factor released by PVAT, through a Ca^{2+}-dependent mechanism, to induce vasodilation of surrounded blood vessels [74]; nevertheless, the nature of ADRF has been long debated [17]. The volatile gaseous mediator H$_2$S, which mediates the anti-contractile effect by opening KCNQ channels in VSMCs, was once suggested to be a primary candidate of ADRF [75]. In the cardiovascular system, H$_2$S is extensively synthesized by cystathionine gamma lyase (CSE) encoded by *CTH* gene in both endothelial cells and perivascular adipocytes [76, 77]. Presence of CSE inhibitors DL-propargylglycine (PPG) and β-cyano-L-alanine (BCA) significantly attenuated the anti-contractile effect of PVAT in rat aortae ex vivo [78]. However, Gollasch et al. recently showed that CSE-derived H$_2$S is unlikely to be the universal ADRF among different species since CSE inhibition by PPG and BCA only reduced the anti-contractile effect in rat but not in mouse aortae [79]. Lack of CSE also did not influence the anti-contractile effect of PVAT in mice [80]. Furthermore, H$_2$O$_2$ is a reactive oxygen species (ROS) generated in both endothelium and PVAT either by spontaneous dismutation of superoxide (O$_2^-$) or dismutation of O$_2^-$ by superoxide dismutases [72, 81]. Co-incubation of PVAT-intact rat aortae with catalase (a H$_2$O$_2$ scavenger) results in an augmented vasoconstriction, indicating the anti-contractile property of H$_2$O$_2$ [71]. Unlike endothelium-derived H$_2$O$_2$ which may induce either vasodilation or vasoconstriction depending on the concentration, vessel type, and species [82, 83], there is so far

Fig. 2 PVAT candidates.
Perivascular adipocytes secrete a
constellation of candidate
molecules for autocrine/
paracrine/endocrine regulation of
various cellular (e.g., adipocyte
formation, browning of WAT-like
adipocytes), physiological (e.g.,
vascular tone regulation, lipid
metabolism), immune (e.g.,
monocyte/macrophage infiltra-
tion, inflammation) and patho-
logical events (e.g., vascular dys-
function, hypertension, athero-
sclerosis and coronary heart dis-
ease). Perivascular adipocytes
might be a potential source of cir-
miRNAs for long-distance endo-
crine regulation
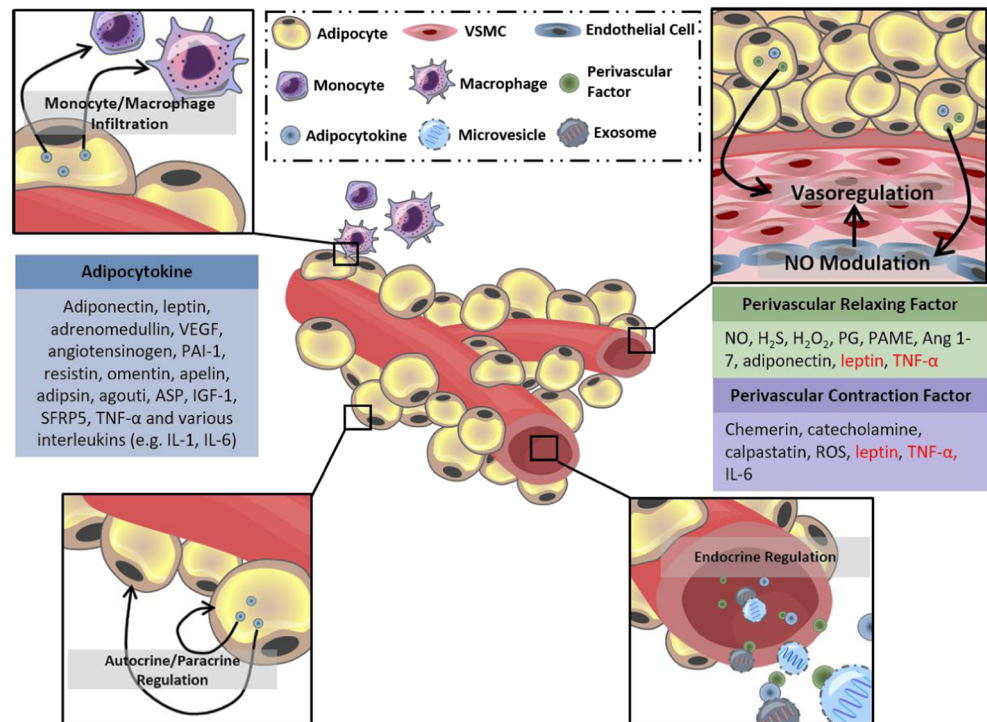
only experimental evidence supporting the anti-contractile
property of PVAT-derived H_2O_2 via the activation of soluble
guanylyl cyclase (GC) within VSMCs [71, 72].

Earlier than the discovery of endothelium-derived NO, the
lipid compound prostaglandin (PG) was identified in 1977 to
be one of the first critical modulators of vascular function in
both physiological and pathophysiological states [84].
Synthesized by two cyclooxygenase (COX) enzymes
(COX1 and COX2) from arachidonic acid, the five primary
PGs bind to specific PG receptors to either induce vasodilation
[prostaglandin D2 (PGD_2); prostaglandin I2 (PGI_2)] or vaso-
constriction [prostaglandin F2α ($PGF_{2\alpha}$); thromboxane A2
(TXA_2)] or both [prostaglandin E2 (PGE_2)] [85]. Similar to
that in endothelium, expression of both COX1 and COX2 in
PVAT bestows the ability to generate PGs, especially the
vasodilatory PGI_2 [86]. Through binding to the prostacyclin
receptor (IP) on VSMC, endothelium-derived PGI_2 stimulates
G_s subunit to activate adenylate cyclase for higher cyclic aden-
osine monophosphate (cAMP) production [87]. Subsequently,
the adenosine triphosphate (ATP)-sensitive potassium chan-
nels (K_{ATP}) and delayed-rectifier potassium channels (K_{dr})
of VSMC sense the increase in intracellular cAMP levels,
resulting in their opening/activation, responsible for
prostacyclin-mediated vasodilation [88]. However, although
it was shown that PGI_2 from PVAT improves HFD-impaired
endothelial function of ApoE$^{-/-}$ mouse carotid artery [30],
whether PVAT-derived PGI_2 follows the same mechanistic
aspect as endothelium-derived PGI_2 remains is yet to be de-
termined. In contrast, PVAT releases another lipophilic and
heat-stable factor named methyl palmitate or palmitic acid

methyl ester (PAME), which is associated with a more well-
defined anti-contractile mechanism [89]. PVAT-derived
PAME opens the voltage-gated potassium (K_v) channels on
VSMCs to promote vasorelaxation [33]. In SHR, both anti-
contractile effect of PVAT and secretion of PAME were found
to be diminished, indicating a possible role of PAME in the
pathophysiology of hypertension [33]. More recent studies
demonstrate that PAME could contribute to PVAT-related re-
laxations by activating KCNQ (Kv7) channels in rat aortae but
not in human mesenteric arteries [90].

The RAS of PVAT gives rise to the production of the
heptapeptide Ang1-7, which serves as a vasodilator [91].
Particularly in the aortae and mesenteric arteries of humans
and rodents, PVAT expresses the main RAS components, in-
cluding angiotensinogen, angiotensin-converting enzyme
(ACE), ACE2, renin, (pro)renin receptor, aldosterone, angio-
tensin II type 1 (AT1), and angiotensin II type 2 (AT2) [10,
92]. The RAS components of PVAT enable the conversion of
Ang II to Ang1-7 via ACE2 [10], the relaxing factor that
induces NO release through activating eNOS in endothelial
cells [93]. Therefore, inbalanced release of Ang II and Ang1-7
from PVAT is closely related to the pathophysiology of vas-
cular dysfunction and hypertension [94].

Apart from PVRFs, PVAT also produces PVCFs to modu-
late vasoconstriction. Imbalance between PVRFs and PVCFs
deteriorates vascular function, which is often observed in
pathological conditions such as obesity and hypertension
[95]. Expressed by PVAT, the PVCF chemerin executes the
chemerin-axis by docking to the primary chemerin receptor 23
(ChemR23) on VSMCs, leading to augmented

vasocontraction, particularly in obesity [96]. In addition, norepinephrine (NE) released by PVAT also accounts for the enhanced vasoconstriction [17]. In 2014, Ayala-Lopez et al. showed that the non-sympathetic components within PVAT microenvironment might supply catecholamines in a tyramine-sensitive manner, causing arterial contraction [97]. Later in 2015, the same group provided additional experimental evidence to suggest that PVAT adipocyte expresses organic cation transporter 3 (OCT3) to serve as the prime candidate transporter, responsible for the uptake of NE into PVAT [97]. In other words, the local stores of NE (as catecholamine) in PVAT adipocyte contribute to the arterial contraction [97]. Owen et al., through comparative proteomic analyses on coronary PVAT between lean and obese swine, uncovered that increased contractile effects of obese coronary PVAT are associated with the alterations in the PVAT proteome, particularly the protein calpastatin encoded by the calpastatin (CAST) gene [98]. In the same study, ex vivo verification shows that coronary adipose-derived calpastatin dose-dependently increases contractions of swine coronary arteries in the presence of PVAT [98], providing hints that calpastatin might be a novel PVCF. Moreover, PVAT adipocytes also generate ROS, specifically superoxide anion (O_2^-), by nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase similar to endothelial cells [99]. Through the activation of tyrosine kinase and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway, O_2^- was found to be the augmenting factor of arterial contractile response in rat superior mesenteric artery upon electric field stimulation (EFS) [100].

Notably, PVAT secretes another critical subtype of vasoregulatory candidates, namely adipokines including adiponectin, leptin, TNF-α, and IL-6. Adiponectin is an abundant adipocyte-derived plasma protein which is reported to elicit vasoprotective benefits influencing the function of nearly all cell types within the vessel wall [101]. Basically, adiponectin exerts protective effects on endothelium by activating the AMP-activated protein kinase (AMPK)-eNOS pathway to elevate NO bioavailability and by improving the redox state in vessel through inhibiting eNOS uncoupling, a state when eNOS generates O_2^- instead of NO under oxidative stress [37, 102]. These findings are consistent with several longitudinal studies that high plasma adiponectin levels are associated with a lower risk of cardiovascular events, including acute myocardial infarction and acute coronary syndrome [11]. Leptin is another crucial adipokine mainly produced by adipocytes proportionally to body fat mass [103]. Leptin elucidates vasoregulatory effect through both endothelial-dependent and endothelial-independent manners [104]. In short, leptin can directly stimulate the production of endothelial NO via the AMPK/protein kinase B (Akt)/eNOS signaling cascade to cause peripheral arterial relaxation [105] and can indirectly trigger vasoconstriction via the central activation of

sympathetic nervous system [106]. Leptin resistance during obesity and hyperleptinemia are correlated with hypertension and coronary artery disease [11, 107].

The pro-inflammatory cytokines, such as TNF-α and IL-6, are also bioactive molecules related to vasoregulation. Depending on the pathophysiological conditions, the dual-acting TNF-α can be either a vasodilator or vasoconstrictor [17]. Normally, TNF-α-mediated vasorelaxation involves NO and prostaglandin production [108, 109]. However, under conditions like lack of regular exercise, overnutrition, aging, and smoking, TNF-α production is significantly upregulated , which eventually reduces NO bioavailability by inhibiting eNOS activity and aggravating superoxide generation by NAD(P)H oxidase [110]. Moreover, via the inhibition on cytochrome P450 (CYP 450), TNF-α diminishes epoxyeicosatrienoic acid (EET), one of the endothelium-dependent hyperpolarizing factors (EDHF) [111]. As a consequence, the VSMCs fail to hyperpolarize, resulting in diminished relaxation [112]. Meanwhile, PVAT-derived IL-6 binds to corresponding IL-6 receptor (IL-6R) to cause endothelial dysfunction by reducing bioavailable NO [113]. In opposite, IL-6 can also upregulate TNF-α expression in endothelial cells, but not VSMCs, to restrain NO production [114].

Adipocytokines

Adipocytokines, referred to the constellation of both adipokines and inflammatory cytokines, are important candidates for maintaining the metabolic homeostasis of healthy subjects. Deficiencies in these factors, either owing to adipocyte dysfunction or excess adiposity, are intimately correlated with the pathogenesis of multiple diseases related to obesity, especially CVDs [115]. PVAT secretes an array of adipocytokines, including adiponectin, leptin, adrenomedullin, vascular endothelial growth factor (VEGF), angiotensinogen, plasminogen activator inhibitor-1 (PAI-1), resistin, omentin, apelin, adipsin, agouti, acylation stimulation protein (ASP), insulin-like growth factor-1 (IGF-1), secreted frizzled-related protein 5 (SFRP5), TNF-α, and various interleukins (e.g., interleukin 1 (IL-1), IL-6) [17, 115, 116]. Not only these factors play an autocrine role in the metabolism of PVAT adipocyte itself, they also carry out paracrine/endocrine function to regulate other cellular processes.

In addition to the beneficial effect on vascular tone, adiponectin exerts many other vasculoprotective and angiogenic properties. In terms of ischemia, adiponectin improves angiogenic repair in adiponectin-knockout (KO) mice by activating AMPK signaling in ischemic adductor muscle [117]. In addition, another study using adiponectin-KO mice showed that adiponectin suppresses the neointimal thickening after artery injury [118]. Furthermore, adiponectin performs anti-inflammatory action by inhibiting the production of cysteine-X-cysteine (CXC) receptor 3 chemokine ligands in

macrophages and by reducing the recruitment of T lympho-cytes in atheromata using apoE/adiponectin double-deficient (apoE$^{-/-}$APN$^{-/-}$) mice [119]. Similarly, leptin also contributes to other aspects of cardiovascular system. Structurally similar to the helical cytokine family, leptin acts upon different im-mune cells, including monocytes/macrophages, T cells, and neutrophils to induce the release of inflammatory cytokines like TNF-α and IL-6, promoting vascular inflammation [120, 121]. Another study uncovered that via ERK1/2 and nuclear factor kappa-light-chain-enhancer of activated B cell (NF-κB) pathway, leptin can expedite VSMC proliferation, which ag-gravates arterial intimal thickening and vascular remodeling [122]. Furthermore, PVAT also expresses the angiogenic fac-tor VEGF, which promotes the vasculogenesis and angiogen-esis of endothelium, hematopoiesis of stem cells, and migra-tion of monocytes/macrophages [123]. VEGF was shown to induce BAT-like phenotype in WAT in vivo [124], which ac-counts for the BAT-like properties of PVAT in an autocrine/ paracrine manner. These experimental evidences shed light on the importance of PVAT, which acts as an important source of adiponectin, leptin, and VEGF apart from BAT and WAT.

The multiple PVAT-derived adipokines are crucial in main-taining the homeostasis of cardiovascular system by endocrinal regulation of different cell types. For instance, resistin promotes SMC proliferation and upregulates mRNA abundance of fatty acid-binding protein in endothelial cells to affect blood pressure [125]. Meanwhile, visfatin is considered one of the important VSMC growth factors via nicotinamide mononucleotide (NMN)-mediated ERK1/2 and p38 signaling [126], whereas omentin suppresses neointimal formation after arterial injury, myocyte apoptosis, and VSMC proliferation via AMPK-dependent signaling [127]. Apelin is crucial in modulating cardiac development, vasomotor tone, and angio-genesis [128], and IGF-1 exerts cardioprotective effects by inhibiting oxidative stress and apoptosis of cardiomyocytes [129]. PVAT-derived angiotensinogen is the critical raw mate-rial for RAS to synthesize Ang II, which induces gene reprogramming or necrosis in cardiac myocytes, and upregula-tion of fibrosis-associated genes in cardiac fibroblasts during cardiac dysfunction [130]. During obesity, high circulating level of adipose tissue-derived PAI-1 potentially stabilizes the fibrin matrix by suppressing plasminogen-mediated fibri-nolysis to contribute to atherosclerotic plaque development and/or to promote the uptake of low-density lipoprotein (LDL) into lesion [131].

In healthy subjects, PVAT derives numerous anti-inflammatory adipocytokines, such as adiponectin, SFRP5, and adrenomedullin [11, 132]. Adiponectin, through binding to the COOH terminal of adiponectin receptor (AdipoR) of monocytes/macrophages, activates the AMPK phosphoryla-tion to suppress I kappa B kinase (IKK)-NF-κB pathway, which hinders pro-inflammatory cytokine production to re-duce the risk of acute myocardial infarction and

atherosclerosis [133]. SFRP5 exerts an antagonistic effect against wingless-related MMTV integration site 5A (Wnt5a) signaling, in which Wnt5a exerts pro-inflammatory effects on endothelial cells and pro-atherogenic effect in aortic regions [120, 134]. Meanwhile, the bioactive peptide adrenomedullin was shown to combat inflammation by inhibiting the release of pro-inflammatory cytokines (IL-6, TNF-α, and interleukin 1β (IL-1β)) from lipopolysaccharide (LPS)-activated macro-phages [132, 135]. In contrast, dysfunctional PVAT derives multiple pro-inflammatory adipocytokines, including mono-cyte chemotactic protein-1 (MCP-1), leptin, TNF-α, IL-1β, and IL-6 [136]. During obesity, increased fat mass is associ-ated with the alterations in cellular composition, metabolome, and dysregulated adipocytokine secretome, characterized by dysfunctional adipose tissue [137]. In obesity, dysregulated adipocytes including perivascular adipocytes express classical macrophage features, characterized by a secretome of elevated pro-inflammatory and reduced anti-inflammatory adipocytokine contents (e.g., adiponectin, SFRP5) [136, 138]. Besides, these pro-inflammatory adipocytokines are re-sponsible for prompting a chronic low-grade inflammation state, endothelial cell proliferation, microvascular remodeling, recruitment of macrophages (CD68+ cells) and T cells to ath-erosclerotic lesions, and polarization of macrophages towards M2 phenotype during atherosclerotic progression [11, 139].

miRNAs

The small non-coding miRNAs are important guide molecules in RNA silencing of gene expression [140]. miRNAs are cru-cial in regulating adipogenesis (formation of adipocytes), met-abolic homeostasis, and endocrine functions of adipocytes [141]. Many miRNAs have been identified to be differentially regulated during adipogenesis in human and murine, includ-ing let-7c, miR-143, miR-210, miR-221, miR-27, and miR-30a-e [142]. Notably, obesity alters the expression profile of miRNAs in adipose tissue, influencing the progression of in-flammation in human adipose tissue [141, 142]. For instance, the expressions of miR-26b, miR-132, and miR-155 are asso-ciated with the number of macrophages infiltrating the fat depot [143]. During obesity, the expression of miR-132 is down-regulated and its expression level is related to the acti-vation of NF-κB signaling and transcription of MCP-1 and interleukin 8 (IL-8) [141, 144]. More importantly, in addition to the adipogenesis and modulation of inflammation state of adipocytes themselves, different studies reported that adipose tissue is capable of secreting circulating miRNAs (cir-miRNAs) to the bloodstream for endocrine functions. The cir-miRNAs might be trapped within microvesicles or exosomes [27, 29]. Collectively termed as extracellular vesi-cles, microvesicles (around 100 nm to 1 μm in diameter) and exosomes (around 40–100 nm in diameter) are of different sizes and origins [145].

In 2017, Thomou et al. reported that mice with adipose tissue-specific knockout of miRNA-processing enzyme Dicer (ADicerKO) display a significant decrease of circulating exosomal miRNAs and stated that cir-miRNAs should be defined as a previously undescribed form of adipokine [29]. Earlier in 2010, Ogawa et al. provided in vitro data that 3T3-L1 adipocyte-derived microvesicles contain 143 miRNAs in which most of them are adipocyte-specific and that these miRNA-containing microvesicles could be transported into cultured macrophages [146]. In the same study, miRNA-containing microvesicles were identified to be present in rat serum in vivo [146]. With close proximity to the circulatory system, it is therefore reasonable to hypothesize that PVAT might be one of the closest sources of adipose tissue-derived cir-miRNAs, encapsulated in either exosomes or microvesicles, in the endocrine regulation of other tissues. Moreover, Thomou et al. also reported that Dicer-knockout not only modulates fundamental functions of adipocytes like whitening of BAT, but also influences other tissues altering circulatory lipids, glucose uptake, and insulin resistance [29, 147]. These studies might shed light on the therapeutic potential of targeting cir-miRNAs in cardiovascular complications concerning abnormal energy intake and lipid metabolism.

PVAT Innervation

Little is known about the effects of PVAT on nervous systems controlling blood vessels. A recent review in 2014, by Bulloch and Daly, highlighted our poor understanding of the link between PVAT and the autonomic nervous system, or other nerves, that are involved in controlling the vasculature [25]. In fact, the innervation of PVAT was first demonstrated in 1970, when Diculescu and Stoica produced a set of Falk fluorescence plates demonstrating a clear innervation of perivascular fat in the rat mesenteric artery [148]. However, it is only recently that the interest to investigate neurotransmission in PVAT has been revived.

Blood vessels, especially resistant vessels, receive multiple perivascular innervations from autonomic system (sympathetic nerves) and non-adrenergic non-cholinergic (NANC) neurotransmissions, including sensory nerves, peptidergic innervations [calcitonin gene-related peptide (CGRP) and substance P], and nitrergic innervation. In addition, co-transmission concept that includes purinergic signaling and NPY also mediates vascular tone. Purinergic signaling arises from both sympathetic and sensory nerves while NPY is the second main neurotransmitter co-released from sympathetic nerve terminals [149]. Due to limited availability of research articles on neuronal expression and activity within PVAT, the present article can only review sympathetic and sensory neurotransmissions in PVAT. Both sympathetic and sensory nerves are well-known as the key players in vascular

regulation and interact reciprocally [149]. For instance, activation of sensory nerves reduced adrenergic neurotransmission via pre-junctional inhibition and treatment with CGRP or SP decreased the amplitude of neurogenic-evoked vasoconstriction [150, 151]. Conversely, capsaicin-induced transient receptor potential cation channel subfamily V member 1 (TRPV1) desensitization and inhibition of CGRP receptors enhanced neurogenic vasoconstriction [152, 153].

Sympathetic Nervous System Innervation of Adipose Tissue

The innervation of BAT by the sympathetic nervous system (SNS) has long been recognized both at the level of blood vessels and also directly on adipocytes [154]. SNS innervation plays a critical role in regulating non-shivering thermogenesis in rodents by controlling the release of noradrenaline (NA) from sympathetic nerve terminals, which consequently stimulates β_3-adrenoceptors that turns on a cascade of intracellular events ending in activation of UCP1 and triggering BAT thermogenesis [155, 156]. Investigation using histofluorescence, electron microscopy, and unilateral denervation model verified the sympathetic innervation of BAT [155, 157]. Further examination with retrograde viral transneuronal tract tracer, pseudorabies virus, injected into the central sympathetic outflow, revealed that circuits ultimately terminate in BAT, indicating that the SNS outflow directly originates from the brain [155].

Various experimental approaches, including neurochemical (NE turnover), neuroanatomical (viral tract tracing), and functional (sympathetic denervation-induced blockade of lipolysis) studies have revealed SNS innervation in WAT [158–160]. In WAT, the SNS is the principal initiator of lipolysis in mammals [161]. It has been demonstrated that the sympathetic nervous system plays a role in modulating lipolysis in white adipose tissue and the degree of lipolysis appears to be determined by the relative expression of adipose adrenoceptors [158]. Adipocytes express receptors for the sympathetic co-transmitters NPY and ATP (NPY receptors and purinoceptors, respectively) and also express muscarinic receptors and receptors for peptides including angiotensin II and SP [162]. Four adrenoceptor subtypes, α_2-, β_1-, β_2-, and β_3-adrenoceptors, exist in WAT [163–165], in which all beta (β_1-, β_2-, and β_3-) adrenoceptors stimulate lipolysis while α_2-adrenoceptors inhibit the process [166]. The SNS innervation in WAT also plays a role in the control of lipid proliferation [158, 167]. It has been shown that sympathetic denervation of WAT in vivo stimulates fat cell proliferation [160].

SNS Innervation of PVAT

In nearly every vascular bed studied across different animal species, the expression of sympathetic nerves has been shown

and it accounts for the largest proportion of innervation in the resistance vasculature [149]. Sympathetic nerves mainly regulate medium and small arteries, and innervation is sparser in large conduit arteries [168]. It has been reported that vascular responses to sympathetic nerve activity (SNA) can vary, depending on the content and composition of vesicles released from specific axon varicosities [169, 170], the size and location of vessel branches within resistance networks [171, 172], and the frequency and firing pattern of action potentials [173].

In 2011, Dashwood and Loesch showed that PVAT of human saphenous vein receives direct sympathetic innervation using immunostaining technique [174]. More recently, Bulloch and Daly also provided evidence of sympathetic innervation in PVAT, of mouse mesenteric arteries, using immunofluorescence techniques [25]. Adipocytes were observed to be innervated by sympathetic nerves, with mast cells commonly located along the nerves [148]. The same study also found that BAT comprises more nerve fibers compared to WAT [148]. Consistently, another study found that adrenergic nerves innervated only 2–3% of all WAT isolated from rats mesentery, epididymis, omentum, and subcutis which were fed, fasted or fasted, and then refed [175]. Although it is becoming more apparent that sympathetic nerves are distributed within PVAT, however, its influence on vasoregulation is not well-defined yet.

In the seminal study of PVAT in 1991, the potential involvement of sympathetic nerves in modulating contractile response to electrical field stimulation has been revealed [32]. Electric field stimulation (EFS) produced a frequency-dependent contraction in intact rat aortae but no response observed in cleaned tissues [32]. The authors postulated that this was most likely due to the presence of sympathetic nerves in the PVAT [32]. In 2006, Gao et al. revisited the neurogenic contractile response in tissues with PVAT and their studies were carried out in rat mesenteric arteries [100]. The group produced a similar observation to that of Soltis and Cassis, in which PVAT augmented the arterial contractile response to perivascular nerve stimulation [32, 100]. This was shown to occur through the production of superoxide mediated by NAD(P)H oxidase and involved activation of tyrosine kinase and MAPK/ERK pathways [100]. In 2010, Lu et al. further explored a pro-contractile property of PVAT on perivascular nerves by providing evidence for the presence of angiotensin II in mesenteric PVAT [176]. This PVAT-derived compound was suggested to potentiate EFS-evoked neurogenic contractile responses in isolated rat mesenteric arteries, consistent with previous observations that angiotensin II enhances sympathetic neurotransmission through pre- and post-junctional mechanisms [177, 178].

Despite the majority of studies showing that sympathetic activation in PVAT promotes vasoconstriction in different vessels [32, 100, 176], however, there are few recent studies that contradict the role of PVAT-derived sympathetic nerves in vascular tone regulation. Ayala-Lopez et al. demonstrated that removal of PVAT did not reduce response of EFS stimulus at 20 Hz of rat aortae; however, the presence of PVAT in rat superior mesenteric artery potentiated a 20-Hz-induced contraction [97]. The authors also showed that PVAT comprises functional catecholamines and which are independent of sympathetic nerves and tyramine-sensitive [97]. In a more recent publication, the same group also found that PVAT of human splanchnic blood vessels contain elements of an adrenergic system with capabilities of synthesis and storage of catecholamines, which may influence vascular tone [179]. However, how PVAT catecholamines would be stimulated or released physiologically demands explanation. Recently, Saxton et al. also supported the role of PVAT as a reservoir for NE; however, the group suggested that these catecholamines are released from sympathetic fibers via organic cation transporter 3, thereby preventing NE from reaching the blood vessel and causing contraction [180]. In contrast to previous studies, the group showed that the presence of PVAT potentiated vasorelaxation of healthy C57BL/6J mouse mesenteric resistance arteries [180]. The study suggests that electric activation of sympathetic nerves in PVAT stimulates the release of a transferable anti-contractile factor (adiponectin) and the release of the adiponectin is dependent on β3-adrenoceptor activation [180]. Torok et al. compared the effect of PVAT on sympathetic neurotransmission in normotensive and hypertensive rats [181]. The group demonstrated that PVAT exhibited inhibitory influence on the contractions to endogenous norepinephrine released from arterial sympathetic nerves during EFS in superior mesenteric artery isolated from normotensive Wistar-Kyoto rats (WKY), which is in line with a recent study [180]. However, the anti-contractile effect against sympathetic-induced vasoconstriction was abolished in spontaneous hypertensive rats [181]. In contrast, the abdominal aorta with intact PVAT elicited larger contractions to EFS and tyramine when compared to the aortae after removing PVAT [181]. The authors attributed that the difference in the modulatory effect of PVAT on adrenergic contractions between abdominal aortae and superior mesenteric arteries might be due to the endogenous NE of PVAT [181].

Accumulating data on the effect of sympathetic neurotransmission in PVAT marks discrepancy. The diversity of experimental conditions, species, and vessels used may account for this contradictory observations. As for experimental conditions, many studies used different parameters (voltage, frequency, duration, and pulse) for studying direct adrenergic activation. Some studies used supramaximal voltage, although majority of studies used nerve blockers such as guanethidine and/or tetradotoxin to verify nerve mediation in EFS-evoked responses. It has been proposed that greater voltage potentiates EFS-enhanced vasoconstriction in PVAT-intact preparations, probably due to generation of superoxide anions [180]. Furthermore, certain groups carried out experiments under

basal condition while some other groups conducted experiments under raised tone, using different agents. The conflicting data should be addressed and the role of sympathetic innervation in PVAT should be elucidated as it may represent a novel target in the treatment of cardiovascular diseases.

The Interaction Between Sympathetic Nerves and PVAT-Derived Mediators

PVAT is well-recognized as a source of numerous vasoactive compounds. It therefore holds promise as a novel therapeutic target. Despite this, information on the link between PVAT-derived mediators and the neurovascular system is sparse. To date, very few in vitro studies that have investigated specifically on the relationship between PVAT-derived nerves and PVAT mediators [180, 182], although there are substantial number of studies which have examined the non-PVAT-specific interaction between sympathetic activation and adipokines. Receptors for adrenergic system and its co-transmission have been detected in adipocytes and stimulation of these receptors modulate adipocytes physiology (e.g., lipolysis and lipogenesis) and contents [162]. Hence, it is postulated that sympathetic innervation in PVAT modulates PVAT mediator release and integral for normal vascular function as well as pathophysiology and vice versa. Indeed, our understanding on the complex interaction between PVAT-derived nerves and PVAT-derived factors is at its infancy, despite it has huge potential in modulating vascular tone. A challenge to scrutinize the crosstalk includes the extremely short duration of neurotransmission and the involvement of non-neuronal effect resulting from the overstimulation of SMCs. In addition, technique to elucidate the PVAT-specific interaction in vivo proves to be challenging and still have not been developed yet.

Recently, it has become apparent that the sympathetic system is a key regulator of leptin production in WAT [183]. Sympathomimetic amines and cold exposure or fasting (which results in sympathetic stimulation in WAT) decrease leptin gene expression in the tissue and thus leptin production [183]. Furthermore, it has been shown that the β3-adrenoceptor is involved in inhibiting insulin-stimulated leptin secretion from isolated rat adipocytes [184], and it has also been postulated that the sympathetic system might contribute to supporting an inhibitory action on leptin synthesis [183]. Conversely, sympathetic blockade often increases circulating leptin and leptin gene expression [183]. In turn, leptin regulates arterial pressure by enhancing sympathetic nerve activity to brown adipose tissue, kidney, and other tissues [185]. Acceleration of sympathetic activity is a hallmark of obesity. In obese patients, it has been shown that cytokines derived from adipocytes such as leptin, TNF-α, and IL-6 mediate sympathetic activation [186]. Some studies indicated that leptin acts both centrally and peripherally to potentiate the

sympathetic nervous system, including enhancing catecholamine synthesis in the adrenal medulla [187, 188], which subsequently activate the renal sympathetic nerves, hence increase blood pressure. Resistin, a pro-contractile adipokine, acts directly in the central nervous system to influence the sympathetic nerve activity [189]. There is growing evidence that resistin may have complex interactions with leptin [189, 190]. Both of these adipokines synergistically excite sympathetic nerves innervating cardiovascular organs but could antagonize each other's actions on BAT, in which resistin inhibits BAT activity and vice versa [189].

Adiponectin, a protective adipokine, has been shown to be regulated by adrenergic system. Imai and co-researchers demonstrated that sympathetic nervous system suppresses serum adiponectin levels and adiponectin mRNA expression in mice subcutaneous, epididymal, and mesenteric WAT in vivo [191], and the regulation differs among WAT depots of mice. In their study, it was shown that cold exposure suppresses serum adiponectin levels through sympathetic nerve activation [191]. Consistently, in healthy male volunteers, exposure to cold for 2 h activated sympathetic nerves, which consequently reduced adiponectin release [192]. Nonetheless, subcutaneous adipokine gene expression was unaltered [192]. The same study found that sympathetic stimulation triggered MCP-1 release [192]. On the contrary, a very recent study showed that direct sympathetic stimulation in PVAT of mice mesenteric arteries in vitro enhanced the release of adiponectin via β3-adrenoceptor activation [180].

IL-6 is a pro-inflammatory cytokine shown to be secreted in higher concentrations from PVAT compared with other fat depots and potentially has a role in promoting arterial stiffness [193, 194]. An in vivo study by De Luigi et al. showed that sympathectomy increased lipopolysaccharide-induced IL-1β and IL-6 messenger RNA in rat adrenals and spleen and concluded that sympathetic nervous system denervation enhances the synthesis and production of peripheral IL-1β and IL-6 by central lipopolysaccharide [195]. Sympathetic neurons have also been shown to stimulate production of IL-6 in vitro using cell culture [196]. MCP-1, another pro-inflammatory cytokine, has been reported to be rapidly expressed by sympathetic ganglion neurons following axonal injury [197].

Sensory Innervation in Adipose Tissue

Sensory nerves are abundant in WAT [158, 160]. Sensory innervation of WAT was first shown neuroanatomically using an anterograde tract tracer, true blue, which resulted in labeled neurons in the dorsal root ganglia (DRG) [198]. Further experiments including histology and a transneuronal viral tract tracer, the H129 strain of herpes simplex virus-1 (HSV) [199], convincingly corroborated the existence of CGRP and SP containing nerves within WAT. BAT also has significant

sensory innervation, as revealed by immunohistochemical staining for CGRP and SP [154, 200].

There is accumulating evidence that BAT has marked sensory innervation, as shown by immunohistochemical markers of sensory neuropeptides such as CGRP and SP [155]. Denervation of sensory nerves in BAT results in general decreases in BAT growth and protein content, mitochondrial content such as cytochrome oxidase activity, and thermogenic capacity, such as UCP1 content [201, 202]. Collective data obtained from different experimental protocols including anterograde viral tract tracer [198], co-culture of DRG cell and adipocytes, and immunohistochemistry staining strongly indicate that WAT is innervated by sensory nerves [203]. Recently, Murphy et al. showed endogenous leptin secreted from WAT activates spinal sensory nerves innervating Siberian hamster brain in a paracrine manner [204]. Despite the innervation and feedback loops of sensory nerves that have been recognized in WAT [205], the significance of the innervation is still poorly understood and warrants further exploration.

Sensory Neurotransmission in PVAT

Evidence has accumulated that NANC nerves play important roles in regulating vascular tone [206]. In NANC neurotransmission, sensory nerves play a vital role in vasoregulation and generally cause vasodilatation [206, 207]. Sensory nerves demonstrate physiological antagonism of sympathetic nerve-dependent vasoconstriction [208] and also possesses both anti-dromic and orthodromic conduction; thus, this allows their participation in local axon reflexes independent of efferent signaling from the cell body [209]. CGRP, a potent vasodilator, has been recognized as the primary neurotransmitter of sensory nerves while SP is a cotransmitter [207, 210]. CGRP is a 37-amino acid neuropeptide that is primarily localized to C and Aδ sensory fibers and has two major forms, α and β [211]. αCGRP is predominantly expressed in the nervous system while β-CGRP is primarily expressed in the enteric sensory system [212]. A recent study has provided solid evidence that sensory nerves are intimately linked to the sympathetic and angiotensin signaling systems [211]. In CGRP knockout mice, enhanced hypertension and vascular remodeling/inflammatory changes in an angiotensin II-induced hypertension model have been demonstrated [213]. Hence, this has created a concept that sensory nerves appear to play a protecting role against the adverse effects associated with the cardiovascular disturbance.

In vascular arena, PVAT has now has become the subject of intense investigation as it involves in cardiovascular pathophysiological conditions. In spite of the extensive research on PVAT, surprisingly, expression and role of sensory nerves in PVAT have not been described previously. It was thought that sensory nerves which involved in blood vessel regulation were only confined at the adventitia and relaxation responses

to EFS in rat isolated mesenteric artery were credited to myogenic tone or dependent on an intact endothelium [214, 215]. Nonetheless, it is very intriguing to find out that there is a mismatch of EFS experiment outcomes for isolated rat mesenteric arterial bed and rat isolated mesenteric artery. Furthermore, evidence of sensory-induced vasorelaxation in other isolated arteries is limited [182]. In rat PVAT-intact mesenteric artery beds, EFS elicited robust sensory neurogenic relaxation responses but these responses were markedly reduced in PVAT-denuded mesenteric artery beds or abolished in clean rat mesenteric artery [182]. The discovery made by Abu Bakar et al. on the expression of sensory nerves within PVAT and functional sensory nerves that are critically dependent on PVAT has orientated our understanding on the role of sensory nerves [216]. PVAT-denuded vessels that are normally used in conventional vascular experiments have led to a postulation that sensory nerves are not involved in vasodilatation of rat mesenteric artery; however, this concept has been refuted by the recent finding [182]. On contrary to EFS-induced sensory stimulation, it is interesting to note that PVAT removal does not totally eradicate EFS-evoked frequency-dependent vasoconstriction (sympathetic activation) [180, 181], although some studies demonstrated significant reduction of EFS-evoked sympathetic vasoconstriction in clean vessels [32, 100, 176]. In addition, while many studies have shown extensive sensory innervation at the adventitia of clean vessels [182, 207, 217], however, it is unclear why vasodilatation cannot be demonstrated in the absence of PVAT. The data however further corroborates that PVAT plays a crucial role in sensory-induced vasodilation.

In comparison to sympathetic nerves which have been located in PVAT in 1970 [148], it is only recently that sensory nerve innervation has been demonstrated in PVAT [182]. A double staining protocol with the presence of anti-CGRP and anti-protein gene product (PGP) 9.5 antibodies was applied to verify the observation [182]. The distribution of nerves was found to be more extensive in small arteries (second-order of mesenteric artery) compared to large arteries (superior mesenteric artery), which is consistent with EFS data which showed neurogenic relaxation responses to EFS were greater in second-order mesenteric artery compared to superior mesenteric artery [182]. Interestingly, cell bodies or neuronal somata were visualized in PVAT of rat mesenteric artery [182]. The observation is not in line with the traditional notion, in which cell bodies of CGRP were believed to be located only in DRG [218], and terminate peripherally on blood vessels and other tissues innervated by the sensory nervous system, and also centrally in laminae I/II of the dorsal horn of the spinal cord [219]. However, a more recent research using DNA sequence analysis to show 100% homology with the β-CGRP cDNA indicates that mRNA encoding β-CGRP is expressed in the vessel, exhibited the presence of adventitial neuronal somata in small rat mesenteric artery [220], which is consistent with

the recent observation [182]. CGRP immunoassay study data further supports the existence of sensory nerves in PVAT layer [182].

Investigation to elucidate the interaction between PVAT-derived sensory nerves and PVAT-derived mediators has been attempted in the recent study [182]. The attempt has led to a development of a new technique which exclusively study the interaction between PVAT-derived nerves and PVAT-derived factors; tissues (mesenteric artery beds) in the presence or absence of PVAT were perfused under normal or low oxygenation, EFS was applied and samples (perfusates) were collected during EFS (very short period), and the samples were then subjected to multiplex assay for adipokines (adiponectin, leptin, IL-6, MCP-1, TNF-α, interleukin beta (IL-β), and total PAI-1 analysis [182]. Under standard oxygenation, only leptin level was shown to be elevated in PVAT-intact preparations [182], which suggests that there is a link between endogenous leptin and sensory nerves. Presumably, CGRP and/or other neurotransmitter(s) released from sensory nerves during EFS act at receptors on the adipocytes in PVAT to cause a release of leptin. Furthermore, exogenous leptin potentiated neurogenic-mediated relaxations in mesenteric arteries [182]. This observation is consistent with leptin's effect in the cardiovascular system. Under normal conditions, leptin demonstrates vasorelaxing effect [221, 222]. The anti-contractile effect of leptin is achieved via both endothelial-dependent (through involvement of NO) and endothelial-independent mechanisms [223, 224]. It has been shown that leptin dilates canine small mesenteric arteries and veins by a mechanism involving endothelial release of nitric oxide [221]. Recently, the role of leptin secreted from WAT as a paracrine factor to activate spinal sensory nerves has been shown in Siberian hamsters [204]. A more recent publication shows that leptin receptors *LepR* are expressed on sensory neurons, and the deletion of leptin signaling in vagal afferent neurons results in hyperphagia and obesity [225]. Hence, the present data adds a new insight on the relationship between leptin and sensory nerves.

The release of leptin was virtually abolished by both PVAT removal and under conditions of low oxygen. In addition, low oxygen levels were shown to enhance inflammatory mediator, IL-6 release [182]. The observation highlights that the level of oxygen plays a role in mediating the release of some PVAT mediators. Moreover, acute hypoxia model that was employed in the study might be extrapolated for certain disorders, such as hypoxia in obesity, and can be exploited for studying the effect of PVAT on neurotransmissions under pathological conditions.

Future Perspectives—What Is Next?

The autocrine/paracrine/endocrine functions of PVAT have been elucidated by an abundance of experimental and clinical research. However, there still remain some major unanswered questions and emergent future directions about PVAT. In the first place, the true identity of ADRF still requires confirmation. Although certain studies pointed that H_2S might potentially be the primary ADRF candidate of perivascular adipocytes [75], however, H_2S might not be the universal ADRF since CSE inhibition does not diminish the anti-contractile effect in mouse aortae [79], unlike endothelium-derived NO which is the universal vasodilator of different species. Furthermore, there is data indicating that adiponectin is not the ADRF using an approach of utilizing adiponectin gene-deficient mice [226]. Another investigation provided clues that the deficiency of leptin receptors in the Zucker fa/fa rat showed no significant modification on the anti-contractile effect of PVAT, implying that leptin probably is not the ADRF [227]. Despite the unclear chemical nature of ADRF, a promising ADRF candidate should at least fulfill the requirement to mimic ADRF characteristic effects on K^+ channels (i.e., it should be a potent opener of KCNQ and probably other K_v channels), as reviewed in 2012 and 2017 [6, 17]. Therefore, future studies need to explore the constellation of ADRF-activated KCNQ channels and other involved K_v channels and to identify the identity of ADRF with reference to the stated requirements using gene-silencing or knockout techniques.

In addition, more efforts are needed to perform comparative studies to dig out the similarities between the mechanisms of endothelium-induced and PVAT-induced relaxation, for the purpose of reductionism. For example, further experiments should be carried out to verify whether PVAT-derived PGI_2 follows the same mechanistic aspect as endothelium-derived PGI_2. Multiple rodent models are currently available, characterized by the reduction in the content of BAT and/or WAT (Table 2). However, the lack of report on the actual reduction of PVAT might limit the dependability of certain models such as transgenic skinny and FAT-ATTAC mice [54, 56]. Hence, further verification on the PVAT content of the genetic engineered mice might extend the generality of PVAT studies.

In 2017, Thomou et al. provided experimental clues in hinting the novel endocrine regulatory approach of adipose tissue via cir-miRNAs [29]. The close proximity between PVAT and vascular tree provides sufficient suspects that PVAT might be one of the major sources of cir-miRNA factory. However, the field concerning PVAT-derived cir-miRNA is still largely undefined so we propose the following questions: (1) whether PVAT shares similar miRNA expression profile as BAT or WAT, (2) whether exosomes and microvesicles cir-miRNAs are the sole packaging approaches of cir-miRNAs for long-distance metabolic regulation, (3) what is miRNA profile of extracellular vesicles, and (4) how the onset and progression of CVDs are mediated by cir-miRNAs. According to the genome-wide comparative

expressional studies between subcutaneous and coronary perivascular adipose tissue-derived adipocytes, a signature of 307 differentially expressed genes for cardiovascular homeostasis was identified [7]. It is of rationale to hypothesize that PVAT should have different miRNA expression profile and hence altered cir-miRNA expression profile. Apart from exosomal and microvesicular cir-miRNAs, whether adipose tissue generates other carriers for cir-miRNA packaging, such as microparticles, requires further confirmation. Previous study showed that adipocytes are able to release microparticles, which act as "find-me" signals to prompt macrophage migration [228], and therefore microparticles might be a third option of miRNA carrier. Past investigation focused on how the generation of fibroblast growth factor 21 is affected by the presence of cir-miRNAs [29]; therefore, future studies can concentrate more on the role of cir-miRNAs in cardiovascular complications such as endothelial dysfunction, coronary heart disease, and atherosclerosis.

Conclusions

Since the identification of anti-contractile effect of PVAT in 1991, more and more primary and clinical investigations unravel the roles of PVAT in addition to the vasoregulatory effect. Accompanied by the increasing rates of obesity worldwide, more scientists and clinicians are now aware of the pivotal endocrine and paracrine roles of adipose tissue in obesity-related cardiovascular events. More importantly, the close proximity between PVAT and vascular system facilitates the interaction between PVAT and vascular system. PVAT, through the production of numerous perivascular contracting/relaxing factors and adipocytokines, acts as an autocrine/paracrine/endocrine regulatory center for the homeostasis of cardiovascular system, sharing the status not less important than that single layer of endothelium. Furthermore, PVAT provides an interface for active PVAT-neuronal communications and may be a potential source of cir-miRNAs. Therefore, PVAT is a master endocrine organ, much more than a mechanistic support to the vasculature.

Compliance with Ethical Standards

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