THE IMPACT OF ADIPOSE TISSUE DYSFUNCTION ON CARDIOVASCULAR AND RENAL DISEASE

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THE IMPACT OF ADIPOSE TISSUE DYSFUNCTION ON CARDIOVASCULAR AND RENAL DISEASE

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EDITORIAL
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Editorial: The Impact of Adipose Tissue Dysfunction on Cardiovascular and Renal Disease

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Keywords: abdominal aortic aneurysm (AAA), PVAT, perivascular adipose tissue, obesity, vascular, vascular diseases, adipose tissue

Editorial on the Research Topic

The Impact of Adipose Tissue Dysfunction on Cardiovascular and Renal Disease

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Obesity is considered an adverse metabolic disease with high risk of vascular complications, including cardiovascular disease (CVD) and chronic kidney disease (CKD). These obesity-related complications are strongly related to excess adipose tissue accumulation, with earlier studies centered on the impact of typical visceral versus subcutaneous adipose tissue accumulation. However, the adipose tissues around blood vessels and organs, including perivascular adipose tissue (PVAT), pericardial adipose tissue (PAT), epicardial adipose tissue (EAT) and perirenal adipose tissue (PRAT), have attracted a great deal of attention recently. Dysfunction in these "novel" adipose tissues have been associated with greater risk of CVD and CKD in obesity than in traditional visceral adipose tissue. Therefore, studies on the interactions of various adipose tissue malfunctions to metabolic-related diseases are vital for improved prevention and treatment of cardiometabolic and renal diseases. This Research Topic aimed to provide a platform for novel advances in dysfunction of various adipose tissues to identify significant contributors to metabolism-related vascular complications, especially CVD and CKD. The Research Topic represents a collection of 7 review articles and 3 original research articles ranging from basic studies to clinical trials, which provide insights into better understanding of the pathophysiology of adipose dysfunction in CVD and CKD.

PVAT, a fat pad surrounding the blood vessels, maintains normal vascular function. However, PVAT becomes dysfunctional in obesity and other pathological conditions. The review by Chen et al. summarizes recent findings regarding dysfunction and inflammation of PVAT in vascular activity, vascular aging, hypertension, atherosclerosis, diabetes and a few other pathological conditions. This review article highlights how PVAT malfunction promotes vascular diseases through stimulation of inflammatory factors and various secreted adipokines, and that

anti-inflammatory therapy to mitigate PVAT inflammation may be a valuable target for future treatment of vascular diseases.

Abdominal aortic aneurysms (AAAs) are irreversible vascular diseases with high mortality rates associated with aneurysm rupture. The association between PVAT and AAA development was reviewed by Ye et al. Factors derived from PVAT are involved in all stages of AAA disease development, including inflammatory cell infiltration, the onset of oxidative stress, and matrix metalloproteinase activation. Excessive adipocyte accumulation derived from PVAT in ruptured AAA walls is closely linked with AAA progression. Through discussion of what is known about PVAT-derived factors, this review highlights the need for additional studies into identifying therapeutic drugs targeted for PVAT dysfunction to reduce AAA rupture-induced mortality.

PVAT dysfunction following vascular damage also promotes neointimal formation by releasing adipocytokines that can regulate the phenotypic switch of vascular smooth muscle cells. Original research studies by Lei et al. found that MIA3/TANGO1 regulates vascular remodeling response to injury. MIA3/TANGO1 deficiency inhibits neointimal formation by preventing vascular smooth muscle cell proliferation/migration, ameliorating neointimal hyperplasia, and maintaining PVAT function during injury-induced vascular remodeling. MIA3/TANGO1 may be a novel potential target for neointimal formation after vascular injury.

Given the interactions between adipose and CVD, several authors centered their work on the cross-talk between the adipose tissue surrounding the heart or myocardium (PAT or EAT) and coronary artery disease (CAD). Using a variety of bioinformatics algorithms, Li et al. highlighted potential roles of molecular alterations in PAT and its association with CAD. 147 differently expressed genes and altered predicted pathways were identified to be mainly associated with regulation of immune system and inflammatory response in PAT of patients with CAD. With the outbreak of coronavirus disease 2019 (COVID-19), patients with metabolic disease and high adverse cardiovascular events attracted attention. The review by Lasbleiza et al. emphasized the harmful role of excess EAT on myocardial injury among COVID-19 patients with obesity. This inflamed EAT depot may participate in COVID-19-related cardiac injury due to its unique anatomical contact with the myocardium and its inflammatory status. The impact of adipose tissue dysfunction on CVD was also reviewed by Bermúdez et al. This review discussed how "sick" adipose tissue affects cardiac pathology, such as arterial fibrillation, coronary artery disease, and myocardial infarction. Changes in adipose tissue microenvironment and metabolic reprogramming in adipose tissue were also summarized in this article.

PRAT, a fat pad surrounding the kidney, has recently been implicated in the regulation of kidney function. In a Mini-Review, Hammoud et al. presented a general overview of new insights linking CVD and CKD, focusing on metabolic disturbances affecting the physiological function of PRAT and potential mechanisms. The review summarized PRAT regulation in various metabolic states and CKD and strengthened the vital role of PRAT, a usually neglected adipose tissue, on regulating homeostasis.

The gut microbiome has also emerged as a critical regulator of host metabolism. It possesses specific impacts on systemic metabolisms and CVD. Yang et al. reviewed the role of gut microbiota and its metabolites in the development and pathogenesis of CVD caused by adipose tissue dysfunction and potential targeted therapies against undesirable gut microbiota. The authors also summarized the present state of clinical therapies for adipose tissue dysfunction targeting the gut microbiota.

Sestrin2, a highly conserved stress-induced protein, may represent a novel antioxidant target for metabolic diseases. Gong et al. include a balanced and comprehensive view of specific mechanisms of Sestrin2 actions in the development of different diseases. This review briefly introduced the potential for Sestrin2 as a clinical marker or therapeutic target. It primarily summarized the regulatory interactions between Sestrin2 and AMPK/mTOR signaling and the effects of Sestrin2 on glucose and lipid metabolism, aging and myocardial energy metabolism.

Telomere shortening and telomerase activity caused by inflammation and oxidative stress are also associated with cardiovascular risk. Li et al. investigated the telomerase RNA component (TERC), telomerase reverse transcriptase (TERT) gene variants, and acute heart failure (AHF) in a prospective study that enrolled 322 patients. The authors found that seven single nucleotide polymorphisms (SNPs) of TERC and TERT are independent risk factors for predicting 18-month mortality in AHF.

Overall, the articles outlined above in this Research Topic underscore the critical role of various adipose tissues dysfunction in CVD and CKD progression, and poses a timely question of whether targeting specific adipose tissue depots may become an important target for better, more efficacious approaches to prevent and/or treat CVD and CKD.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved it for publication.

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ORIGINAL RESEARCH
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Prognostic Association of TERC, TERT Gene Polymorphism, and Leukocyte Telomere Length in Acute Heart Failure: A Prospective Study

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Background: Telomere length and telomerase are associated in development of cardiovascular diseases. Study aims to investigate the associations of TERC and TERT gene polymorphism and leukocyte telomere length (LTL) in the prognosis of acute heart failure (AHF).

Methods: Total 322 patients with AHF were enrolled and divided into death and survival group according to all-cause mortality within 18 months. Seven single nucleotide polymorphisms (SNPs) of TERC and TERT were selected. Baseline characteristics, genotype distribution and polymorphic allele frequency, and genetic model were initially analyzed. Genotypes and the LTL were determined for further analysis.

Results: Compared to carrying homozygous wild genotype, the risk of death in patients with mutated alleles of four SNPs- rs12696304(G>C), rs10936599(T>C), rs1317082 (G>A), and rs10936601(T>C) of TERC were significantly higher. The dominant models of above were independently associated with mortality. In recessive models, rs10936599 and rs1317082 of TERC, rs7726159 of TERT were independently associated with long-term mortality. Further analysis showed, in haplotype consisting with TERC - rs12696304, rs10936599, rs1317082, and rs10936601, mutant alleles CCAC and wild alleles GTGT were significant difference between groups ($P<0.05$). CCAC is a risk factor and GTGT is a protective factor for AHF patients. Relative LTL decreased over age, but showed no difference between groups and genotypes.

Conclusions: The SNPs of TERC and TERT are associated with the prognosis of AHF, and are the independent risk factors for predicting 18-month mortality in AHF.

Keywords: telomere—genetics, telomerase reverse transcriptase (TERT), genetic polymorphism, heart failure, prognosis, telomerase RNA component (TERC)

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INTRODUCTION

Heart failure (HF) is a series of symptoms and signs caused by structural and/or functional abnormalities of the heart. Acute heart failure (AHF) is defined as a rapid onset of new or worsening of HF, which often a potentially life-threatening condition requiring immediate assessment and treatment (1). The incidence of heart failure is closely related to age and affected by the environment and the interaction of multiple genes (2). Cardiovascular disease accounted for more than 40% of the deaths for residents (3) despite the treatment advancement. Screening of high-risk patients would significantly reduce the mortality rate of patients with acute heart failure and also save medical expenses.

Aging, inflammatory response and oxidative stress are the main endogenous factors causing changes in telomere shortening and telomerase activity. Telomere shortening is associated with cardiovascular risk factors such as age, gender, smoking, sedentary lifestyle, obesity, excessive drinking, and psychological stress (4–6). In addition, atherosclerosis, essential hypertension, heart failure, coronary heart disease and other cardiovascular diseases are also accompanied by changes in telomere length and telomerase activity (7–10).

Telomeric DNA sequences of the same species are highly conserved. The length of telomere DNA gradually shortens with aging and cell division, which as a circadian clock and eventually initiate apoptosis (11, 12). Among, leukocyte telomere length (LTL) has been recognized as a clinical indicator for measuring the risk of age-related diseases. Animal studies have shown that telomere depletion is associated with apoptosis in cardiomyocytes and chronic heart failure (CHF) (13, 14). A follow-up study in CHF patients showed the shorter telomere length was associated with higher mortality and rehospitalization rate as well (15).

Genome-wide association studies (GWAS) (16–18) have demonstrated that differences in telomere length (TL) between individuals may be associated with single nucleotide polymorphisms (SNPs), of which five loci are involved in telomere biology, including chromosomes 3q26.2 (TERC), 5p15.3 (TERT), 4q32.2 (NAF1), 10q24.33 (OBFC1), 18 and 20q13.3 (RTEL1).

Telomerase is a ribonucleoprotein polymerase and composed of the telomerase reverse transcriptase (TERT), the telomerase RNA component (TERC), and the TERC-binding protein dyskerin, which plays a key role in the regulation of telomere length (TL). Changes in TL and telomerase activity are the potential pathological features of the above age-related conditions. In such pathological conditions, TERT and TERC are considered to participate in abnormally enhanced local or systemic oxidative stress of the telomere erosion and attrition. However, there is lack of study regarding the prognostic effect of TL in the acute setting of heart failure.

This study aims to further explore the polymorphisms of telomerase gene TERC and TERT, in the leukocyte telomere length regulation and the relation with the prognosis of acute heart failure.

METHODS AND MATERIALS

Participants

This study prospectively enrolled 322 patients whom hospitalized for AHF in Cardiology Department of the First Affiliated Hospital with Nanjing Medical University from March 2012 to April 2016. Inclusion criteria were age ≥18 years, with new-onset AHF or acute exacerbation of chronic heart failure. All patients received standard treatment after admission according to the guideline (19). Patients with malignant tumor, severe mental illness, and/or uncontrolled systemic disease were excluded. The study protocols were approved by the independent Ethics Committee (First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China). Each participant had signed informed consent. The trial was registered at http://www.chictr.org.cn/(Trial registration: ChiCTR—ONC-12001944, Registered 5 Feb 2012, http://www.chictr.org.cn/showprojen.aspx?proj=7604).

Data Collection and Follow-Up

Within 24 h admitted to the hospital, all patients underwent comprehensive clinical evaluation included demographics, physical examination, laboratory results, clinical data, medical history and etiology of AHF.

All venous blood samples were obtained at the admission or in the following morning and analyzed in the central laboratory of our hospital to measure the complete blood count and other biochemical markers. Transthoracic echocardiography (TTE) was used for evaluating the left ventricular systolic and diastolic function on the Vivid E9 ultrasound system (GE Medical System, United States of America).

The primary endpoint was all-cause mortality during the 18-month follow up. Patients were evaluated for the primary endpoint by out-patient visit, telephone evaluation, and/or confirmation of their family or physician every 3 months. Patients were separated into survival group and death group according to the primary endpoint.

Single Nucleotide Polymorphism Selection

The genes were selected using the genome database of Chinese Han Beijing (CHB) population and review of previous relative studies literature related to association between TERC and TERT polymorphisms (20–26). 7 SNPs were selected in for further analysis including TERC (rs12696304, rs10936599, rs1317082, rs10936601, rs16847897) and TERT(rs7726159, rs2736100). The minor allele frequencies (MAF) of all the selected SNPs were greater than 5%.

Genotyping and Leukocyte Telomere Length Measurement

Genomic DNA was extracted from whole blood samples using the TIANamp Blood DNA Kit (DP318; TIANGEN, Beijing, China) and the concentration was measured by spectrometry (NanoDrop 2000 spectrophotometer, Thermo Scientific, Waltham, MA, USA).

TaqMan fluorescent probe quantitative PCR technology were used for the SNP genotyping. Based on the nucleic acid sequences of the seven selected SNPs, the allele-specific TaqMan probes were designed, synthesized and verified by Thermo Fisher Scientific. Reaction system included TaqPath ProAmp Master Mix 2.5 μl, Assay Mix Probe [20×] 0.25 μl, Genomic DNA/Nuclease-Free Water (ddH2O) 2.25 μl (5 ng/ul). Genotyping were performed using ABI PRISM 7900HT Sequence Detection System 2.4 (SDS2.4) in accordance with the manufacturer's protocol.

Genotyping quality control procedures leading to SNP exclusion were call rate <90% and P<0.05 for deviations from Hardy-Weinberg equilibrium (HWE). The selected SNPs in the study were successfully genotyped with 99.68% of call rate. With 36B4 for internal control and ETV6 as primer, each LTL sample was measured using a multiplex quantitative real-time PCR method and was calculated by T/S ratios (T, telomere signal; S, single copy gene signal) (27–29).

Statistical Analysis

Continuous variables were expressed as the mean ± standard deviation (SD) and compared by student's t-test or one-way ANOVA for normal distribution, or expressed as median with inter-quartile range (IQR) and compared by Mann-Whitney U test or Kruskal-Wallis H test for skewed distribution. Categorical variables and frequency of events were reported as numbers (percentages) and compared by chi-square test. In the correlation analysis, after the logarithmic transformation of the data that skewed distribution, the Pearson method is used for the analysis. The online SHESIS software (http://analysis.bio-x.cn/myAnalysis.php) was used to analyze the HWE, genotype, allele frequency distribution, linkage disequilibrium and SNP haplotypes (30, 31). Kaplan-Meier and multi-variable COX analysis was used to analyze the prognosis of AHF patients under different genetic models of SNPs. Correlation analysis between haplotype and AHF prognosis was performed, the P value was subjected to FDR (False Discovery Rate) correction and Bonferroni correction.

All statistical analyses were two-sided and the significance level was set to $P < 0.05$. When $D' > 0.8$ and $r^2 > 0.33$, linkage disequilibrium (LD) was considered between sites. SPSS version 19.0 statistical package (SPSS, Chicago, IL, USA) and Microsoft Excel were used for all statistical analyses.

RESULTS

Baseline Characteristics

In total, 322 AHF patients were divided into death group (80 cases) and survival group (242 cases). The detail characteristics of the participants between two groups were shown in **Table 1**. There were 15 variables from the baseline characteristics (**Table 1**) were considered to be statistically significant. Variables included systolic and diastolic blood pressure, aspartate aminotransferase (AST), albumin (ALB), Serum creatinine (Scr), blood urea nitrogen (BUN), uric acid (UA), CystatinC (CysC), serum potassium (K), serum sodium (Na), hemoglobin (HB), D-Dimer, NT-proBNP, pulmonary artery systolic pressure (PASP), and comorbidities of renal dysfunction (All P<0.05). In addition, there was no significant difference in sex distribution, treatment regimen, NYHA classification, and other comorbidities (All P>0.05).

Distribution of the Genotypes and Allele Frequencies

The distribution of genotypes and allele frequency (**Table 2**) of the seven SNPs of TERC and TERT genes were consistent with HWE in the death group and survival group of patients with acute heart failure (P>0.05), indicating sample has a population representative.

Among which the genotype distribution and polymorphic allele frequencies of the four loci of TERC gene were statistically different between the two groups (P<0.05):

rs12696304 (G/C, Hazard Ratio - HR=1.82, 95% CI: 1.25–2.63, P=0.0016);

rs10936599 (T/C, HR=1.87, 95% CI: 1.31–2.69, P=0.0006);

rs1317082 (G/A, HR=1.92, 95% CI: 1.34–2.76, P=0.0004);

rs10936601 (T/C, HR=1.82, 95% CI: 1.25–2.63, P=0.0016);

The genotype distribution and polymorphic allele frequency of the other three SNPs (rs16847897, rs7726159, and rs2736100) showed no significance between groups (P>0.05).

Comparison of the Single Nucleotide Polymorphism Genotype Under Different Genetic Models

Genetic model analysis showed the genotype distribution and comparison between the death and survival groups of the seven selected SNPs of TERC and TERT genes under different genetic models are shown in **Table 3**.

i. Both the dominant and recessive model genotype of rs10936599 (CC+TC *vs.* TT, HR:2.84 [1.48–5.44]; CC *vs.* TT+TC, OR:1.98 [1.10–3.57]) in TERC were statistically different between the death group and the survival group (P<0.05).
ii. Both the dominant and recessive model genotype of rs1317082 (AA+GA *vs.* GG, HR:2.89 [1.51–5.54]; AA *vs.* GG+GA, HR:2.10 [1.16–3.80]) in TERC were statistically different between the death group and the survival group (P<0.05).
iii. The dominant model genotype distribution of rs12696304 (CG+CC *vs.* GG, HR:2.33 [1.37–3.97]) in TERC was statistically different between the death group and the survival group (P<0.05).
iv. The dominant model genotype distribution of rs10936601 (CC+TC *vs.* TT, HR:2.20 [1.30–3.74]) in TERC was statistically different between the death group and the survival group (P<0.05).
v. The recessive model genotype distribution of rs7726159 (AA *vs.* CC+CA, HR:1.97 [1.07–3.63]) in TERT was statistically different between the death group and the survival group (P<0.05).

TABLE 1 | Baseline characteristics between survival group and death group.

Characteristic	Totals (n=322)	Survivals (n=242)	Deaths (n=80)	*P*
Age (year-old)	60.51 ± 16.24	59.62 ± 16.41	63.21 ± 15.50	0.086[a]
Sex (F/M)	110/212	76/166	34/46	0.078[b]
BMI (Kg/m2)	24.27 ± 4.53	24.27 ± 4.50	24.26 ± 4.65	0.982[a]
HR (bpm)	86 ± 22	87 ± 22	85 ± 22	0.411[a]
SBP (mmHg)	125 ± 22	128 ± 23	118 ± 17	0.000[a]
DBP (mmHg)	78 ± 15	80 ± 16	74 ± 12	0.000[a]
ALT (U/L)	26.70 (17.73, 46.68)	31.30 (21.80, 49.70)	24.25 (15.83, 58.13)	0.165[c]
AST (U/L)	29.30 (22.90, 44.18)	30.70 (24.00, 43.90)	27.40 (21.70, 46.35)	0.034[c]
ALB (g/L)	37.36 ± 4.67	37.71 ± 4.67	36.27 ± 4.52	0.020[a]
Scr (umol/L)	87.10 (72.05, 109.95)	89.60 (76.00, 110.70)	96.95 (78.63, 137.88)	0.004[c]
BUN (mmol/L)	7.19 (5.82, 9.54)	7.28 (6.17, 8.71)	8.49 (6.14, 10.90)	0.000[c]
UA (mmol/L)	473.0 (382.0, 582.0)	479.0 (388.0, 576.0)	526.5 (446.0, 748.0)	0.029[c]
CysC (mg/L)	1.31 (1.12, 1.63)	1.31 (1.10, 1.57)	1.52 (1.23, 1.77)	0.001[c]
K (mmol/L)	3.99 ± 0.51	3.96 ± 0.48	4.09 ± 0.56	0.042[a]
Na (mmol/L)	139.76 ± 4.09	140.19 ± 3.81	138.49 ± 4.62	0.004[a]
Ca (mmol/L)	2.25 ± 0.14	2.25 ± 0.14	2.25 ± 0.14	0.993[a]
HB (g/L)	133.08 ± 20.81	135.24 ± 20.08	126.55 ± 21.70	0.001[a]
RDW (%)	14.95 ± 4.78	14.75 ± 5.35	15.54 ± 2.29	0.205[a]
D-dimer (mg/L)	0.75 (0.30, 1.72)	0.59 (0.29, 1.58)	0.94 (0.31, 2.84)	0.001[c]
NT-proBNP (ng/L)	1979 (1176, 4315)	1775 (1205, 2933)	2626 (1688, 6615)	0.000[c]
cTnT (ng/L)	61.80 ± 387.34	45.82 ± 295.11	101.74 ± 556.69	0.380[a]
CK-MB (U/L)	35.34 ± 122.90	36.27 ± 139.96	32.98 ± 61.23	0.869[a]
TTE				
LVDd (mm)	61.53 ± 12.51	61.35 ± 11.96	62.08 ± 14.12	0.661[a]
LVDs (mm)	48.85 ± 14.01	48.67 ± 13.52	49.39 ± 15.53	0.697[a]
PASP (mmHg)	42.0 (31.5, 53.0)	40.0 (31.0, 48.0)	48.0 (30.5, 60.5)	0.005[c]
LVEF%	42.50 ± 14.81	42.43 ± 14.57	42.71 ± 15.62	0.885[a]
NYHA				0.228[b]
II (n%)	48 (14.9)	40 (16.5)	8 (10.0)	
III (n%)	181 (56.2)	137 (56.6)	44 (55.0)	
IV (n%)	93 (28.9)	65 (26.9)	28 (35.0)	
Comorbidities				
IHD (n%)	76 (23.6)	55 (22.7)	21 (26.3)	0.545[b]
Cardiomyopathy (n%)	130 (40.4)	101 (41.7)	29 (36.3)	0.431[b]
VHD (n%)	86 (26.7)	63 (26.0)	23 (28.8)	0.663[b]
PHD (n%)	23 (7.1)	17 (7.0)	6 (7.5)	1.000[b]
Atrial fibrillation (n%)	122 (37.9)	94 (38.8)	28 (35.0)	0.596[b]
CHD (n%)	10 (3.1)	6 (2.5)	4 (5.0)	0.273[b]
HTN (n%)	162 (50.3)	125 (51.7)	37 (46.3)	0.440[b]
DM (n%)	77 (23.9)	55 (22.7)	22 (27.5)	0.450[b]
Pulmonary Infection (n%)	66 (20.5)	48 (19.8)	18 (22.5)	0.633[b]
Renal dysfunction (n%)	20 (6.2)	10 (4.1)	10 (12.5)	0.013[b]
Thyroid Dysfunction				0.763[b]
Hyperthyroidism(n%)	7 (2.2)	6 (2.5)	1 (1.3)	
Hypothyroidism(n%)	1 (0.3)	1 (0.4)	0 (0)	
Smoking (n%)	121 (37.6)	98 (40.5)	23 (28.8)	0.064[b]

F, Female; M, Male; BMI, Body Mass Index; HR, Heart Rate; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; ALB, Albumin; Scr, Serum Creatinine; BUN, Blood Urea Nitrogen; UA, Uric Acid; CysC, CystatinC; HB, Hemoglobin; RDW, Red blood cell Distribution Width; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; cTnT, cardiac troponin T; CK-MB, Creatine kinase-MB; LVDd, Left Ventricular Diastolic Dimension; LVDs, Left Ventricular Systolic Dimension; PASP, Pulmonary Artery Systolic Pressure; LVEF, Left Ventricular Ejection Fraction; IHD, Ischemic Heart Disease; VHD, Valvular Heart Disease; PHD, Pulmonary Heart Disease; CHD; Congenital Heart Disease; HTN, Hypertension; DM, Diabetes Mellitus.

[a]Calculated by unpaired t-test; [b]calculated by Chi-square test; [c]calculated by rank sum test.

Association Between Genetic Polymorphisms and Prognosis

To further assess the association between each selected SNP and the prognosis of acute heart failure, Kaplan-Meier curve analysis were used (**Figures 1A–G**). Results showed that the overall survival rate decreased over time.

i. For rs12696304, rs10936599, rs1317082, and rs10936601 of TERC gene, the survival rate of AHF patients carrying mutant alleles were significantly lower than the homozygous wild alleles (P<0.05, **Figures 1A–D**).
ii. Under both dominant and recessive models, rs10936599 (**Figure 1B**) and rs1317082 (**Figure 1C**) were associated with decreased survival in patients with AHF (P<0.05);
iii. Dominant models of rs12696304 (**Figure 1A**) and rs10936601 (**Figure 1D**) were associated with decreased survival rate of AHF patients (P<0.05);

TABLE 2 | Distribution of the genotypes and allele frequencies.

Gene	SNPs	Group	Genotype					HWE		Allele		HR (95%CI)	χ2	P
			MM	Mm	mm	χ^2	P	χ^2	P	M	m			
TERC	rs12696304	D	26(0.329)	41(0.519)	12(0.152)	10.00	0.0068	0.41	0.5244	93(0.589)	65(0.411)	1.81 (1.24–2.63)	9.75	0.0018
	G/C	S	128(0.529)	93(0.384)	21(0.087)			0.48	0.4875	349(0.721)	135(0.279)			
	rs10936599	D	13(0.163)	44(0.550)	23(0.287)	12.17	0.0023	1.1	0.2934	70(0.438)	90(0.562)	1.87 (1.31–2.69)	11.77	0.0006
	T/C	S	86(0.355)	115(0.475)	41(0.169)			0.06	0.8089	287(0.593)	197(0.407)			
	rs1317082	D	13(0.163)	44(0.550)	23(0.287)	13.10	0.0014	1.1	0.2934	70(0.438)	90(0.562)	1.92 (1.34–2.76)	12.75	0.0004
	G/A	S	87(0.360)	116(0.479)	39(0.161)			0.00	0.9744	290(0.599)	194(0.401)			
	rs10936601	D	27(0.338)	40(0.500)	13(0.163)	9.79	0.0075	0.08	0.7775	94(0.588)	66(0.412)	1.82 (1.25–2.63)	9.99	0.0016
	T/C	S	128(0.529)	93(0.384)	21(0.087)			0.48	0.4875	349(0.721)	135(0.279)			
	rs16847897	D	26(0.325)	41(0.512)	13(0.163)	0.60	0.7411	0.22	0.6368	93(0.581)	67(0.419)	0.87 (0.61–1.26)	0.53	0.4674
	C/G	S	90(0.372)	117(0.483)	35(0.145)			0.09	0.7604	297(0.614)	187(0.386)			
TERT	rs7726159	D	27(0.338)	32(0.400)	21(0.263)	5.38	0.0679	3.06	0.0804	86(0.537)	74(0.463)	1.26 (0.88–1.81)	1.64	0.201
	C/A	S	83(0.343)	122(0.504)	37(0.153)			0.51	0.4737	288(0.595)	196(0.405)			
	rs2736100	D	24(0.300)	35(0.438)	21(0.263)	3.41	0.1815	1.23	0.2683	83(0.519)	77(0.481)	0.79 (0.55–1.13)	1.63	0.2022
	A/C	S	78(0.322)	123(0.508)	41(0.169)			0.4	0.525	279(0.576)	205(0.424)			

SNP, single nucleotide polymorphism; HWE, Hardy-Weinberg equilibrium; HR, hazard ratio; D, death group; S, survival group; M, major allele; m, minor allele.

TABLE 3 | Comparison of the SNP genotype under different genetic models between the death and survival groups.

Gene	SNPs	Group	Dominance		HR (95%CI)	χ2	P	Recessive		HR (95%CI)	χ2	P
			Mm + mm	MM				mm	MM + Mm			
TERC	rs12696304	D	53(0.671)	26(0.329)	2.29 (1.34–3.90)	9.53	0.002	12(0.152)	67(0.848)	1.89 (0.88–4.03)	2.74	0.133
	G/C	S	114(0.471)	128(0.529)				21(0.087)	221(0.913)			
	rs10936599	D	67(0.838)	13(0.163)	2.84 (1.48–5.44)	10.5	0.001	23(0.287)	57(0.713)	1.98 (1.10–3.57)	5.26	0.035
	T/C	S	156(0.645)	86(0.355)				41(0.169)	201(0.831)			
	rs1317082	D	67(0.838)	13(0.163)	2.89 (1.51–5.54)	10.9	0.001	23(0.287)	57(0.713)	2.10 (1.16–3.80)	6.17	0.021
	G/A	S	155(0.640)	87(0.360)				39(0.161)	203(0.839)			
	rs10936601	D	53(0.663)	27(0.338)	2.20 (1.30–3.74)	8.83	0.003	13(0.163)	67(0.838)	2.04 (0.97–4.30)	3.65	0.062
	T/C	S	114(0.471)	128(0.529)				21(0.087)	221(0.913)			
	rs16847897	D	54(0.675)	26(0.325)	1.23 (0.72–2.10)	0.57	0.503	13(0.163)	67(0.838)	1.15 (0.57–2.30)	0.15	0.718
	C/G	S	152(0.628)	90(0.372)				35(0.145)	207(0.855)			
TERT	rs7726159	D	53(0.663)	27(0.338)	1.03 (0.60–1.75)	0.01	1.000	21(0.263)	59(0.738)	1.97 (1.07–3.63)	4.89	0.03
	C/A	S	159(0.657)	83(0.343)				37(0.153)	205(0.847)			
	rs2736100	D	56(0.700)	24(0.300)	1.11 (0.64–1.92)	0.14	0.782	21(0.263)	59(0.738)	1.75 (0.96–3.18)	3.35	0.073
	A/C	S	164(0.678)	78(0.322)				41(0.169)	201(0.831)			

SNP, single nucleotide polymorphism; HR, hazard ratio; D, death group; S, survival group; M, major allele; m, minor allele.
The major/minor alleles were G/C (rs12696304), T/C (rs10936599), G/A (rs1317082), T/C (rs10936601), C/G (rs7726159), C/A (rs1317082) and A/C (rs2736100) respectively.

iv. Recessive model of rs7726159 (**Figure 1F**) was associated with a decrease in survival rate in patients with AHF (P<0.05).
v. The other two SNPs—rs16847897 (**Figure 1E**) and rs2736100 (**Figure 1G**) did not show differences in neither the models between the death group and the survival group (P>0.05).

Afterward, univariate COX regression analysis all showed significance in the five SNPs (TERC—rs12696304, rs10936599, rs1317082, rs10936601; TERT—rs7726159). Wild genotype was set as the reference genotype. The association of different genetic models and prognosis of AHF were shown in **Table 4**.

After adjusted with the 15 significant variables in the baseline characters, results showed that for rs12696304, rs10936599, rs1317082, and rs10936601 of TERC, the risk of death carrying mutation alleles were higher than those of wild homozygous genotypes, and remained as independent risk factors in AHF patients. The dominant models of these four SNPs were all independently associated with the risk of death in AHF patients (P>0.05).

Furthermore, the recessive models of rs10936599, rs1317082 of TERC, and rs7726159 of TERT were independently associated with the risk of death in AHF patients (P>0.05).

Haplotype Analysis of Telomerase RNA Component and Telomerase Reverse Transcriptase Genes

The linkage disequilibrium (LD) analysis of five SNPs of TERC and two SNPs of TERT in AHF patients is shown in **Figure 2**. Further haplotype analysis was performed based on the results of linkage disequilibrium analysis.

The four haplotypes consisting of rs12696304 (G/C), rs10936599 (T/C), rs1317082 (G/A) and rs10936601 (T/C) sites, which the overall frequency was above 1%, showed

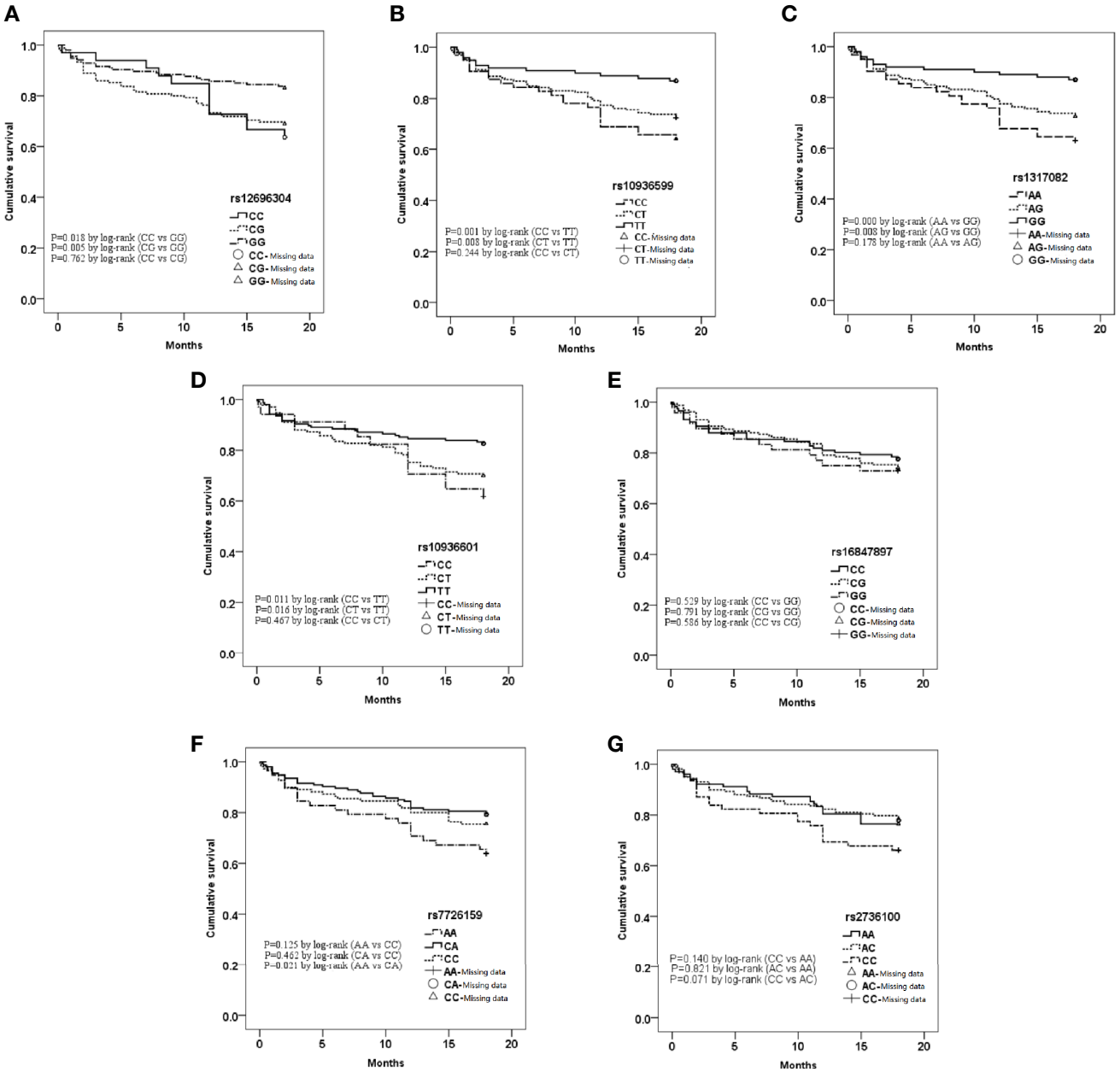

FIGURE 1 | Kaplan-Meier curve analysis of 7 single nucleotide polymorphisms (SNPs) in different genotypes. **(A)** rs12696304; **(B)** rs10936599; **(C)** rs1317082; **(D)** rs10936601; **(E)** rs16847897; **(F)** rs7726159; **(G)** rs2736100.

statistically significant in overall haplotype distribution between the survival and death groups (P=0.0031).

The frequency of haplotype CCAC (H1) was significantly higher in the death group (OR: 1.79 [1.23–2.61], P<0.05); The frequency of haplotype GTGT (H4) was significantly lower in the death group than in the survival group (OR: 0.54 [0.38–0.78], P < 0.05). After corrected by Bonferroni or FDR method, the two haplotypes CCAC and GTGT composed of these four SNPs remained significant (P<0.05, **Table 5**).

The four haplotypes consisting of the rs7726159 (C/A) and rs2736100 (A/C) of TERT gene showed no statistical difference between the groups (P>0.05, **Table 6**).

Leukocyte Telomere Length

Correlation analysis was performed on the difference of LTL between different prognoses and genotypes. There was a significant negative correlation between the relative LTL and age of AHF patients regardless of the primary endpoint (P<0.001,

TABLE 4 | COX regression analysis of different genetic models.

	Genotype		χ^2	HR (95%*CI*)	*P*
rs12696304	Codominance	GG	–	1.0(ref.)	–
		CC	8.16	3.35(1.46–7.66)	0.004
		CG	8.96	2.65(1.40–5.02)	0.003
	Dominance	GG	–	1.0(ref.)	–
		CC+CG	10.82	2.79(1.52–5.16)	0.001
	Recessive	GG+CG	–	1.0(ref.)	–
		CC	–	–	0.071
rs10936599	Codominance	TT	–	1.0(ref.)	–
		CC	11.1	4.98(1.94–12.79)	0.001
		TC	6.95	3.27(1.36–7.89)	0.008
	Dominance	TT	–	1.0(ref.)	–
		CC+TC	9.05	3.72(1.58–8.74)	0.003
	Recessive	TT+TC	–	1.0(ref.)	–
		CC	5.9	2.07(1.15–3.72)	0.015
rs1317082	Codominance	GG	–	1.0(ref.)	–
		AA	12.39	5.35(2.10–13.63)	0
		GA	6.17	3.05(1.27–7.37)	0.013
	Dominance	GG	–	1.0(ref.)	–
		AA+GA	8.76	3.63(1.55–8.51)	0.003
	Recessive	GG+GA	–	1.0(ref.)	–
		AA	8.35	2.35(1.32–4.18)	0.004
rs10936601	Codominance	TT	–	1.0(ref.)	–
		CC	6.58	3.07(1.30–7.23)	0.01
		TC	10.81	3.01(1.56–5.79)	0.001
	Dominance	TT	–	1.0(ref.)	–
		CC+TC	11.91	3.02(1.61–5.66)	0.001
	Recessive	TT+TC	–	1.0(ref.)	–
		CC	–	–	0.16
rs7726159	Codominance	CC	–	1.0(ref.)	–
		AA	6.82	2.69(1.28–5.65)	0.009
		CA	0.68	1.32(0.69–2.53)	0.409
	Dominance	CC	–	1.0(ref.)	–
		AA + CA	–	–	0.12
	Recessive	CC + CA	–	1.0(ref.)	–
		AA	4.47	1.96(1.05–3.65)	0.034

Adjusted with systolic and diastolic blood pressure, aspartate aminotransferase, albumin, Serum creatinine, blood urea nitrogen, uric acid, cystatinC, serum potassium, serum sodium, hemoglobin, D-Dimer, NT-proBNP, pulmonary artery systolic pressure, and comorbidity of renal dysfunction.

Figure 3), and no significant correlation with the clinical baseline (P>0.05, **Appendix 1**). Moreover, there was no significant difference had found in the research SNPs and their genotypes (P>0.05, **Appendix 2**).

DISCUSSION

Previous studies showed that telomerase, in addition to its nuclear-specific telomere elongation, also has extranuclear non-telomere elongation. TERT as the catalysis subunit of telomerase can regulate the level of mitochondrial reactive oxygen (32, 33). The telomere length or telomerase gene TERC and TERT polymorphisms were correlated with age, the morbidity of tumors and chronic cardiovascular diseases (34–42). These past studies were mostly regarding in chronic development diseases, and the results usually showed correlation of TL, SNPs and the morbidity of these chronic conditions. On the other hand, an underlying mechanism of decreased or stayed LT in acute settings (43). The acute stress on the heart induces compensatory mechanisms aimed at preserving TL by upregulating TERT. Despite increases in TERT, TL decreased or stayed the same in early phrase. As the heart disease progresses, however, these mechanisms become attenuated and then exhausted, leading to telomere attrition and overt cardiac failure.

To further explore the prognostic values of seven TERC and TERT genes single nucleotide polymorphisms (SNPs), and leukocyte telomere length (LTL) in AHF. By using both SNP and haplotype analysis method, we analyzed the relationship between TERC and TERT gene polymorphisms and the prognosis AHF from the perspective of epigenetics, avoiding the false negative or false positive results that might be caused by analyzing a single site.

Our results showed the genotypes rs12696304 (G>C), rs10936599 (T>C), rs1317082 (G>A), and rs10936601 (T>C)

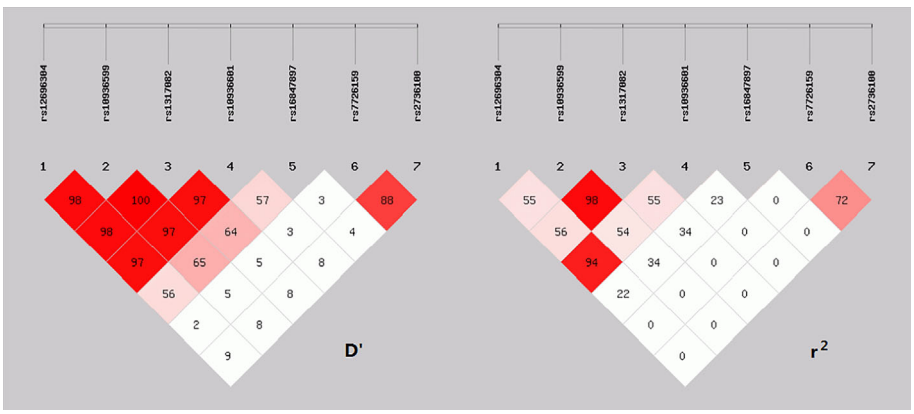

FIGURE 2 | Linkage disequilibrium analysis of 7 SNPs.

TABLE 5 | Comparison of haplotypes with TERC gene.

	Haplotypes	Combinations	Deaths(freq)	Survivals(freq)	χ^2	P	HR (95%CI)	P_b	P_f
TERC	H_1	CCAC	62.97(0.400)	132.99(0.275)	9.219	0.0024	1.79 (1.23–2.61)	0.0096	0.0096
	H_2	CCAT	2.03(0.013)	0.75(0.002)	3.587	0.0583	8.32 (0.97–71.67)	0.2332	0.1166
	H_3	GCAT	22.47(0.147)	59.51(0.123)	0.462	0.4969	1.20 (0.71–2.02)	1.9876	0.6625
	H_4	GTGT	68.50(0.428)	284.48(0.588)	10.826	0.001	0.54 (0.38–0.78)	0.004	0.001
	Global P	–	158	484	11.527	0.0031	–	–	

Haplotypes were in the order of rs12696304(G/C), rs10936599(T/C), rs1317082(G/A) and rs10936601(T/C), respectively.
HR, Hazard Ratio; Pb, Bonferroni adjusted P-values; Pf, False Discovery Rate corrected P-values.

TABLE 6 | Comparison of haplotypes with TERT gene.

	Haplotypes	Combinations	Deaths(freq)	Survivals(freq)	χ^2	P	HR (95%CI)	P_b	P_f
TERT	H_1	AA	7.41(0.046)	10.44(0.022)	2.73	0.0986	2.20 (0.84–5.75)	0.3944	0.3944
	H_2	AC	66.59(0.416)	185.56(0.383)	0.543	0.4611	1.15 (0.80–1.65)	1.8444	0.9222
	H_3	CA	75.59(0.472)	268.56(0.555)	3.284	0.07	0.72 (0.50–1.03)	0.28	0.0933
	H_4	CC	10.41(0.065)	19.44(0.040)	1.685	0.1943	1.66 (0.77-3.61)	0.7772	0.1943
	Global P	–	160	484	6.12	0.106	–	–	

Haplotypes were in the order of rs7726159(C/A) and rs2736100(A/C), respectively.
HR, Hazard Ratio; Pb, Bonferroni adjusted P-values; Pf, False Discovery Rate corrected P-values.

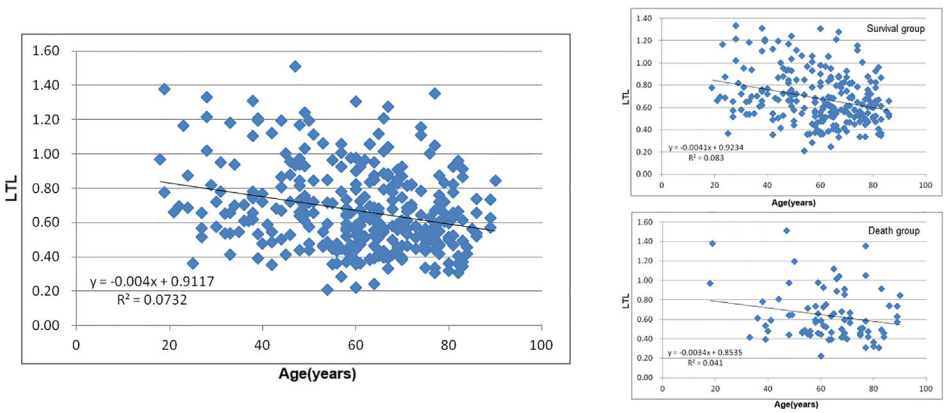

FIGURE 3 | Leukocyte Telomere Length (LTL) in different age and prognosis.

of TERC were the independent risk factors for death in AHF patients after 18 months follow-up, which suggesting these sites can be used to assess the prognosis of patients with AHF.

Haplotype analysis revealed a linkage disequilibrium between the four SNPs above (rs12696304, rs10936599, rs1317082, and rs10936601). The haplotype CCAC consisted of the mutant alleles and the haplotype GTGT consisted of the wild-type alleles of these four SNPs were significant differences between the death group and survival group. Haplotype CCAC is a risk haplotype for patients with AHF, and haplotype GTGT is a protective factor for patients with AHF.

Although the other three selected SNPs did not showed significance between groups; further analysis in the recessive model for rs7726159 of TERT were found related to the prognosis of patients as well. The results of TL analysis showed that there were no significant differences between groups in LTL regarding all genotypes of the seven SNPs in TERC and TERT.

Combined with our results of haplotype analysis, the mechanism might be different in the acute setting of disease. The biological functions and its prognostic influence might not be related directly to LTL, and may be related to its regulation mechanism besides telomere elongation of telomerase. The telomere length shortens in the effects of endogenous factors and attenuates cardiometabolism (44). In AHF, the wild genotype might provide physiological effects in protective regulation pathways by enhanced telomerase activity acting on telomeres. On the other hand, mutant genotype lost the effects and tend to be more susceptible of the endogenous factors, therefore demonstrated a higher mortality.

Although the underlying mechanisms remain to be systematically investigated, this study offers the prognostic factor of AHF from a molecular biology perspective—the effects of TERC and TERT gene polymorphisms in patients with AHF.

Within the selected SNPs, there were five SNPs of TERC and TERT genes showed a significant correlation to the prognosis of AHF. These five SNPs are all located in the non-coding region, where rs10936599 is located in the 5'UTR region, and the remaining four SNPs (rs12696304, rs1317082, rs10936601, and rs7726159) are located in the intron region. The variation of TERC or TERT gene may affect the transcription process, resulting in changing the expression level of the corresponding protein, which may eventually affect the progression of AHF.

Studies showed that LTL and adipose tissue was highly correlated (45). As smoking, sedentary lifestyle, and obesity are also factors associated with an increased burden of inflammation. Similar to TL, adipose tissue are also associated with adverse cardiometabolic risk factors, and often exhibits proinflammatory and prooxidative metabolic changes (46–48), which might associated to the direct damaging effects of adipose tissue on telomeres and the mediation through the expression of corresponding genes, such as TERT and TERC. On the other hand, adipose tissues showed various regulatory effects on cardiovascular system. Among, epicardial adipose tissue (EAT) regulates physiological and pathophysiological processes in the heart. Although not investigated in our study, our findings provide fundamental knowledge regarding TL and AHF; adipose tissue, especially epicardial adipose tissue, might be involved in these pathological mechanisms. TL and telomerase may be attributed to these regulations of metabonomics in adipocyte.

In addition, this study also has certain limitations. Firstly, the study was a single-center study with a relatively small sample size, which needs a larger cohort to further verify the correlation of the above SNPs and the prognosis of AHF patients. Secondly, the biological functions of the above positive SNPs in AHF are still unclear. Lastly, our study SNPs only included limited sites. Further researches regarding wider genome and the association with adipose tissue in heart failure are needed.

CONCLUSION

The results suggest a potential association between TERC, TERT gene variants and AHF. It provided a valuable prognostic information and will better elucidate the genetic and telomeric mechanisms of patients with acute heart failure. Further genomics and lipidomics investigations are needed.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital, Nanjing Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YL participated in the design of the research and drafted the manuscript. IC participated in the design and is a major contributor in writing the manuscript. ZZ participated in the analyses. WY and HZ performed the analysis and interpretation of the study statistic design. YZ, YL, and XZ supervised the study program and method feasibility. QC and XL contributed to the conception and design of the research, and performed critical revision of the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.650922/full#supplementary-material

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REVIEW
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Relationships Between Perivascular Adipose Tissue and Abdominal Aortic Aneurysms

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Abdominal aortic aneurysms (AAAs) are typically asymptomatic, and there is a high mortality rate associated with aneurysm rupture. AAA pathogenesis involves extracellular matrix degradation, vascular smooth muscle cell phenotype switching, inflammation, and oxidative stress. There is increasing evidence of excessive adipocyte accumulation in ruptured AAA walls. These excessive numbers of adipocytes in the vascular wall have been closely linked with AAA progression. Perivascular adipose tissue (PVAT), a unique type of adipose tissue, can be involved in adipocyte accumulation in the AAA wall. PVAT produces various chemokines and adipocytokines around vessels to maintain vascular homeostasis through paracrine and autocrine mechanisms in normal physiological conditions. Nevertheless, PVAT loses its normal function and promotes the progression of vascular diseases in pathological conditions. There is evidence of significantly reduced AAA diameter in vessel walls of removed PVAT. There is a need to highlight the critical roles of cytokines, cells, and microRNA derived from PVAT in the regulation of AAA development. PVAT may constitute an important therapeutic target for the prevention and treatment of AAAs. In this review, we discuss the relationship between PVAT and AAA development; we also highlight the potential for PVAT-derived factors to serve as a therapeutic target in the treatment of AAAs.

Keywords: abdominal aortic aneurysm, perivascular adipose tissue, obesity, vascular, vascular diseases

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INTRODUCTION

Aortic aneurysms are irreversible, permanent manifestations of local vasodilation that can be either thoracic or abdominal; most comprise abdominal aortic aneurysms (AAAs) (1). AAAs are pathological dilations that are 1.5-fold larger than the normal aortic diameter. There is increasing evidence of excessive adipocyte accumulation in ruptured AAA walls (2). These excessive numbers of adipocytes in the vascular wall are derived from perivascular adipose tissue (PVAT); they have been closely linked with AAA progression (3). PVAT, a unique type of adipose tissue, surrounds most blood vessels (4). Recently, PVAT has attracted considerable interest in the context of vascular diseases. The fundamental regulatory role of PVAT in vascular physiology and dysfunction has been reported to affect both dilated and atherosclerotic aortic diseases (5–7).

Additionally, PVAT can secrete various substances, thus promoting expansion of the AAA wall; AAA diameter in the vascular wall has been shown to significantly decrease upon removal of PVAT (8). However, the underlying mechanism by which PVAT contributes to AAAs is unclear. In this review, we focus on PVAT-derived cytokines in the pathophysiological progression of AAAs. Moreover, we highlight the potential for PVAT-derived factors to serve as a therapeutic target in the treatment of AAAs.

CHARACTERISTICS OF AAA

AAAs can occur in any portion of the inferior phrenic aorta, although they frequently occur in the infrarenal abdominal aorta (9). Most AAAs are small and become increasingly prominent over time. Moreover, the risk of rupture increases with increasing aneurysm diameter (10). Age, increased smoking frequency, family history of AAAs, and a high-fat diet have been associated with AAA expansion (11); this type of change is characterized by progressive expansion and weakening of the three layers of the abdominal aorta (i.e., intima, media, and adventitia). The intima is composed of a layer of endothelial cells upon connective tissue, the media comprises vascular smooth muscle cells (VSMCs) embedded in structural proteins, and the adventitia comprises fibroblasts and collagen fibers (12). Damage to any layer of the abdominal aorta will promote AAA progression, frequently leading to rupture-related mortality. This pathological progression involves extracellular matrix (ECM) degradation, vascular smooth muscle cell phenotype switching, inflammation, and oxidative stress. (**Figure 1**) (13–16). AAAs cause more than 15,000 deaths annually in the United States; approximately 25% of patients with aortic rupture can achieve prolonged survival by undergoing surgery (16–18). Currently, AAAs cannot be treated with medication; treatment is limited to surgical repair to prevent disastrous rupture. However, this treatment does not provide substantial benefits to patients with small AAAs (19). Thus, there is a need to develop new therapies to reduce the risk of AAA rupture.

CHARACTERISTICS OF PVAT

PVAT is a metabolically hyperactive tissue that surrounds many large blood vessels except the cerebral vasculature. Typical PVAT includes adipocytes, microvasculature, stromal cells, and inflammatory cells; the specific phenotype depends on anatomical location and varies markedly due to pathogenesis (20). PVAT contains both white and brown adipose tissue. White adipose tissue (WAT) stores energy in the form of triacylglycerols, which can be mobilized through lipolysis during energy expenditure or increased fasting. Additionally, WAT can secrete various hormones, cytokines, and enzymes. These substances derived from WAT are essential to biological processes such as inflammation, metabolism, and vascular homeostasis (21, 22). In contrast, brown adipose tissue (BAT) can generate heat through intracellular lipolysis and the activity of uncoupling protein 1. Intracellular lipolysis produces fatty acids as thermogenic substrates, while uncoupling protein 1 interrupts electron transport during the generation of adenosine triphosphate within the cristae-dense mitochondria in BAT (20, 23). The compositions of WAT and BAT in PVAT vary throughout the human body. PVAT contains mainly BAT in the thoracic aorta and mainly WAT in the abdominal aorta (6, 24, 25). In normal physiological conditions, PVAT releases vasoactive molecules (e.g., hydrogen peroxide, angiotensin, adiponectin,

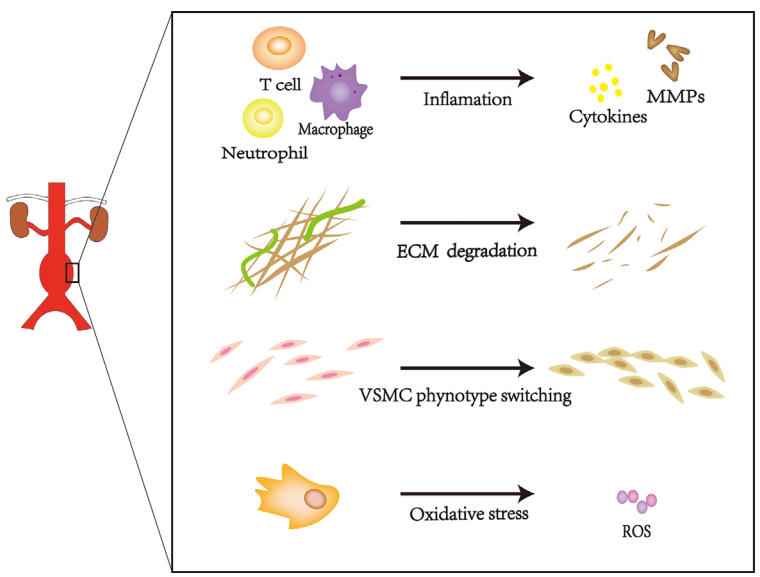

FIGURE 1 | The pathogenesis of AAA.

hydrogen sulfide, and nitric oxide) to attenuate agonist-induced vasoconstriction (26–28). Conversely, PVAT in pathological conditions promotes inflammation and oxidative stress; inhibits the release of vasoprotective adipocyte-derived relaxing factors; and increases the secretion of paracrine factors such as resistin, leptin, cytokines [e.g., tumor necrosis factor α and interleukin (IL)-6] and chemokines [regulated upon activation, normal T cell expressed and secreted (i.e., RANTES) and monocyte chemoattractant protein (MCP)-1] (24, 29, 30). These substances usually contribute to increased incidence of metabolic disease (e.g., obesity, diabetes, and aging), thereby promoting PVAT dysfunction and AAA progression (7, 31). Thus, PVAT has a close relationship with AAAs.

CELLULAR AND MOLECULAR CONTACT BETWEEN DYSFUNCTIONAL PVAT AND AAA PATHOLOGY

There is increasing evidence that dysfunctional PVAT influences AAA progression. This dysfunction involves inflammatory cells (e.g., lymphocyte, macrophages and neutrophil) infiltration and migration from the PVAT to the vascular wall; these cells generate reactive oxygen species to promote elastic arterial stiffness. Furthermore, PVAT can regulate neointimal formation through cytokines that promote VSMC phenotype switching, outer membrane inflammation, and neovascularization. Thus, PVAT-derived biologically active substances may participate in each stage of AAA pathogenesis; targeting these substances may aid in AAA mitigation (**Table 1** and **Figure 2**).

PVAT-Derived Biological Substances in AAAs

In the vascular walls of AAAs, inflammatory cytokines such as MCP-1 and C-reactive protein (CRP) are present at increased levels during pathological conditions (48). Both of these cytokines contribute to elastic arterial stiffness by promoting leukocyte and macrophage adhesion and migration into the vessel wall, leading to VSMC proliferation. These cytokines are secreted at high levels in PVAT (49), where they promote neointimal hyperplasia with macrophage infiltration and vasa vasorum proliferation after vascular damage (32, 33), thereby accelerating aneurysm formation.

Upregulation of MMPs, produced by inflammatory cells and VSMCs, are the basis of AAA pathogenesis. MMPs increase the expression levels of inflammatory substances, which disrupt critical components of ECM (e.g., elastin and collagen fibers) (50–52). ECM degradation by proteolytic enzymes, mainly MMP-2 and MMP-9, reportedly promotes AAA progression (34, 35). Notably, MMP-2 and MMP-9 expression levels are elevated by PVAT-mediated angiopoietin-like protein 2 (Angptl2) (16). Angptl2-deficient mice showed reduced AAA progression compared with wild-type mice; in particular, Angptl2-deficient mice exhibited smaller aneurysms, less vascular structural destruction, and lower MMP expression levels (53). Additionally, Tian et al. constructed wild-type mice with PVAT derived from transgenic mice expressing Angptl2 in adipose tissue; these modified wild-type mice exhibited more frequent neointimal hyperplasia after endovascular injury, compared with wild-type mice that underwent transplantation of wild-type tissue (36). These studies demonstrate that MMP inhibition in PVAT may reduce AAA size, suggesting that AAA progression can be controlled by adjusting PVAT-derived MMPs (54).

Furthermore, although PVAT-derived factors are beneficial in normal physiological conditions, long-term enhancement in pathological conditions may promote disease progression. Platelet-derived growth factor-D (PDGF-D) and vascular endothelial growth factors (VEGF) can repair damaged blood vessels, but both contribute to AAA formation. PDGF receptors are widely expressed in cells of the cardiovascular system, including fibroblasts, smooth muscle cells, and pericytes. PDGF-D signaling has important implications in fibrosis, neovascularization, atherosclerosis, and restenosis (55). A transcriptomics analysis revealed that PDGF-D was strongly expressed in PVAT from obese mice; inhibiting PDGF-D function significantly reduced AAA incidence. The experiment demonstrated that adipocyte-specific PDGF-D transgenic mice were more likely to exhibit AAA formation, accompanied by adventitial fibrosis and inflammation (37). Furthermore, Zhang et al. reported that PDGF-D stimulates the transforming growth factor-beta/small mother against decapentaplegic (i.e., Smad) pathway, thereby mediating AAA formation during obesity

TABLE 1 | Summary of PVAT-derived factors.

Factors	Official full name	Function in AAA	Reference
CRP	C-reactive protein	neointimal hyperplasia	(32, 33)
MCP-1	Monocyte chemoattractant protein-1	neointimal hyperplasia	(32, 33)
MMPs	matrix metalloproteinases	ECM degradation	(34, 35)
Angptl2	angiopoietin-like protein 2	neointimal hyperplasia	(36)
PDGF-D	platelet-derived growth factor-D	adventitial inflammation and fibrosis	(37)
VEGF	vascular endothelial growth factors	adventitial neovascularization	(16)
leptin		vascular remodeling	(38)
T cells	T lymphocytes	Inflammation response	(39–42)
PROK2	Prokineticin 2	inflammation and immune-related processes	(43, 44)
MAP4K1	Mitogen-activated protein kinase kinase kinase kinase 1	inflammation and immune-related processes	(43, 44)
stromal cells		vascular remodeling	(45, 46)
EVs miR-221-3p	extracellular vesicle microRNA-221-3p	VSMC phenotypic switching	(47)

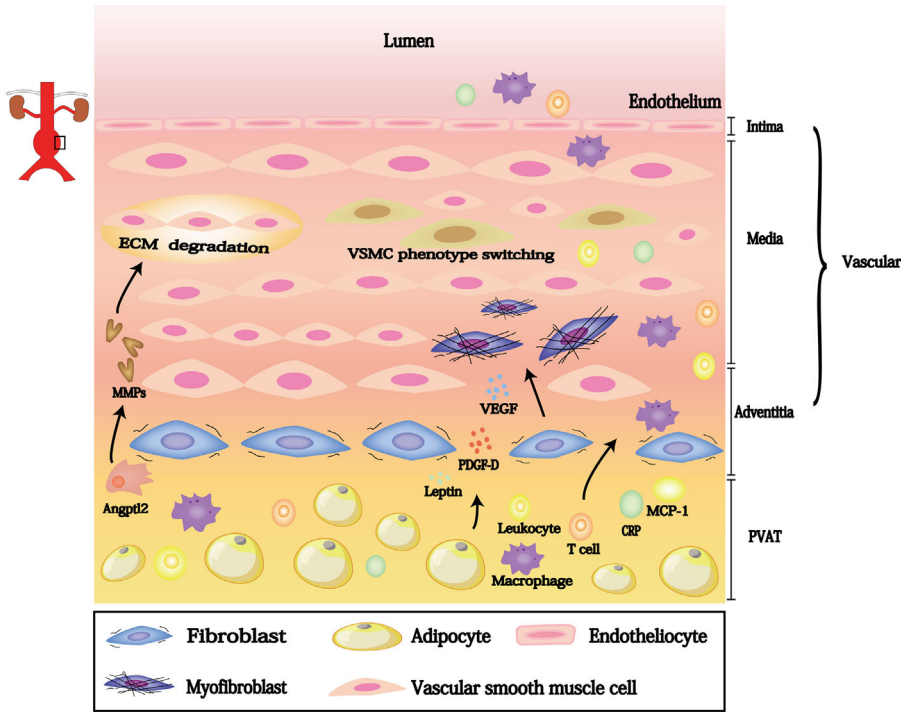

FIGURE 2 | PVAT contributes to the formation of AAA under pathological conditions.

(37, 56). These studies showed that PVAT-derived PDGF-D has a vital role in AAAs. Importantly, PDGF-D stimulates the release of VEGF-A by fibroblasts (57). VEGF-A overexpression in PVAT facilitates adventitial neovascularization; VEGF-A is elevated in aneurysms, compared with non-aneurysmal aortae (16). The inhibition of VEGF-A expression may reduce AAA incidence (58). These findings suggested that PVAT-derived VEGF also has a vital role in AAAs. Therefore, overexpression of PVAT-derived growth factors could contribute to AAA progression by promoting adventitial inflammation.

Leptin is a robustly secreted adipokine with a secretion level directly proportionate to adipocyte size; leptin is closely involved with AAAs (59). Leptin is reportedly increased 60-fold in PVAT from obese mice, and PVAT-derived leptin was twofold greater in AAAs than in normal aortae (60). Chronic elevation of leptin could lead to vasoconstriction and VSMC phenotypic switching (61); both of these changes could accelerate exacerbate vascular remodeling and promote AAA progression (38). Additionally, PVAT-derived leptin participates in AAA pathogenesis through the IL-18 signaling pathway, which involves the IL-18 receptor and NaCl co-transporter (62, 63). Leptin can increase the expression levels of IL-18, IL-18 receptor, and NaCl co-transporter; deletions of these receptors reduced AAA growth (63). These findings suggest that PVAT-derived biological substances contribute to AAA progression.

PVAT-Derived Immune Cells in AAAs

Immune cells from PVAT are also implicated in AAA pathogenesis. T cells are the main leukocyte subset in AAAs. Activated T cells promote the release of pro-inflammatory factors derived from macrophages in AAA models, and their greatest accumulations occur in PVAT (39). Notably, T cells are highly activated in PVAT/vascular walls, and the degree of T-cell infiltration into PVAT is strongly associated with AAA size (39–42). Furthermore, the innate immune signaling molecule CD14 has a vital role in the adventitial recruitment of macrophage precursors, which lead to AAAs; CD14 is reportedly upregulated in PVAT-conditioned medium from an AAA model *in vivo* and *in vitro* (31, 64). Thus, PVAT is a reservoir of T cells and may be critical for modulating the underlying inflammation of AAA (42).

Weighted correlation network analysis showed that prokineticin 2 (PROK2) and mitogen-activated protein kinase kinase kinase kinase 1 (MAP4K1) were hub genes in dilated PVAT samples, where they mediated AAA pathogenesis (43, 65). PROK2 is upregulated in granulocytes and macrophages within inflamed tissue; it reportedly exhibits sevenfold upregulation at AAA rupture sites (66). Furthermore, MAP4K1 expression is increased by T and B cells. Both of these proteins regulate inflammation and immune processes, such as inflammatory cell adhesion, cytokine release, and immune cell activation (43, 44). However, specific mechanisms underlying PVAT-derived gene function in AAAs remain unknown; analysis of these genes may provide promising AAA treatments.

PVAT-Derived Stromal Cells in AAAs

Perivascular adipose tissue-derived stromal cells (PVADSCs) also participate in AAA formation (5, 67). Adipose tissue-derived

stromal cells (ADSCs) are mesenchymal stem cells in essence. Cultured populations of ADSCs contain fibroblast colony-forming units and a proportion of clonable self-renewing cells, but will quit proliferating at less than 20 passages. Thus, it to be more appropriate to use ADSCs (stroma) than the term stem cells for ADSCs (68). PVADSCs can be distinguished into several cell lines under specific culture conditions, including endothelial cells, smooth muscle cells, osteoblasts, and adipocytes (69–71). This capability is particularly robust in young PVADSCs, but is weak in aged cells. Aged PVADSCs show decreased differentiation or aberrant secretion of adipokines and cytokines, which leads to reduction of their protective effects against vascular lesions. These changes could initiate myofibroblast proliferation and migration, followed by neointimal induction (45). Moreover, PVADSCs from AAA patients displayed enhanced senescence manifestation. This manifestation contains increased decreased proliferation, migration ability, mitochondrial fusion, reactive oxygen species production, and decreased mitochondrial membrane potential, which all contribute to AAA formation (46).

PVAT-Derived Extracellular Vesicle miRNAs in AAAs

Multiple types of PVADSCs can be induced by the transfection of microRNA (miRNA) mimics (72). Based on gene set enrichment analysis, the respective expression levels of miR-27b-3p and miR-221-3p in plasma were 1.6-fold and 1.9-fold higher in patients with AAAs than in healthy controls (73). Additionally, miR-221-3p is highly expressed in obese PVAT-derived extracellular vesicles (EVs). PVAT-derived EVs containing miRNAs communicate intercellular messages in AAA pathogenesis (74, 75). Obese mice reportedly secrete large quantities of EVs containing miRNA, which induce inflammatory reactions in PVAT and VSMC phenotype switching in the abdominal aorta. In the context of obesity-associated inflammation, PVAT-derived miR-221-3p could trigger early vascular remodeling (47). Therefore, efforts to target PVAT-derived EVs could provide novel therapeutic approaches for AAAs.

PVAT-TARGETING THERAPY

The only effective therapy against large AAAs or symptomatic aneurysms is open surgery or endovascular repair; however, this provides no clear benefits with respect to small AAAs. Current research regarding drugs and cells aims to identify novel effective therapeutic and preventive strategies for AAAs. Given the roles of MMPs in AAA weakening and rupture, MMPs are considered reliable targets. Some wide-spectrum MMP inhibitors have been developed as therapeutic agents for cancer; however, no trials have shown improved overall survival, and MMP inhibitors can have severe side effects (76). However, a subset of MMP inhibitors may have better effects. In particular, MMP12 is significantly increased in AAAs while peroxisome proliferator-activated receptor γ agonist could reduce MMP12 levels, thus reducing the inflammatory and oxidative statuses of PVAT (77). Furthermore, MMP-targeted imaging can be used to predict AAA progression and rupture risk. Selective MMP12 inhibitors based on 99mTc-labeled radiotracers have the potential for detecting AAA biology and predicting AAA outcome; thus, single photon emission computed tomography imaging research may be useful regarding AAAs (78).

Additionally, some experiments have been conducted to treat AAAs by VEGF or its receptor inhibition (79). VEGF-induced PVAT cell differentiation downregulates protein kinase C epsilon and p21-activated kinase 1 phosphorylation, thus negatively regulating vascular progenitor differentiation (80). Reductions of VEGF signaling-related angiogenesis have been performed to treat AAAs in mice (81). For example, anti-VEGF-A monoclonal antibody suppresses aneurysm development, while receptor tyrosine kinase inhibitor sunitinib limits AAA initiation and progression (79). These findings indicate that VEGF and its receptors have therapeutic potential.

Regenerative medicine has achieved clear therapeutic effects in various cardiovascular diseases, including AAAs. PVADSCs, as immunomodulatory cells, inhibit the activation of T lymphocytes and repolarize the phenotype of M1 macrophages to M2. PVADSCs can differentiate to functional SMC-like cells, but inhibit SMC apoptosis. In addition, PVADSCs produce essential ECM components such as collagen, elastin, and laminin. Experiments involving transplantation of cultured PVADSCs into a mouse vein graft model suggested that PVADSCs promote VSMC differentiation, thereby contributing to vascular remodeling. PVADSCs inhibits high mobility group box 1 release, leading to reductions of proinflammatory cytokines (e.g., IL-17) and protection against AAA formation (82). PVADSCs can maintain a multipotent phenotype and are easily cultured, providing a promising treatment for small AAA (68). Thus, regenerative medicine is a compelling long-term approach for preventing AAA formation. It should be noted that the targeted therapies cannot eradicate the disease, but delay its progression in the initial stages. Therefore, it can only be applied at an early stage of illness. The exact effects still need to be supported by clinical studies.

CONCLUSION

In pathological conditions, PVAT becomes dysfunctional and has a vital role in AAA formation. PVAT-derived factors participate in all stages of pathological AAA formation, including inflammatory cell infiltration, oxidative stress onset, matrix metalloproteinase activation, and VSMC phenotype switching. Thus, PVAT may be a useful new target for the development of AAA therapeutic drugs. Notably, most studies thus far have used *in vitro* and *in vivo* models of AAAs. However, AAA formation in humans is a chronic process. Moreover, the mechanisms that connect PVAT-derived factors and AAAs remain unclear. Additional studies are needed to identify the mechanisms that contribute to AAA inhibition, thus alleviating the risk of AAA rupture-induced mortality and preventing AAA formation.

AUTHOR CONTRIBUTIONS

NH and FH: Conceptualization, Writing - Review & Editing. TY and GZ: Methodology, Software, Visualization, Writing - Original Draft. Other authors: Software. All authors contributed to the article and approved the submitted version.

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MINI REVIEW
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Perirenal Adipose Tissue Inflammation: Novel Insights Linking Metabolic Dysfunction to Renal Diseases

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A healthy adipose tissue (AT) is indispensable to human wellbeing. Among other roles, it contributes to energy homeostasis and provides insulation for internal organs. Adipocytes were previously thought to be a passive store of excess calories, however this view evolved to include an endocrine role. Adipose tissue was shown to synthesize and secrete adipokines that are pertinent to glucose and lipid homeostasis, as well as inflammation. Importantly, the obesity-induced adipose tissue expansion stimulates a plethora of signals capable of triggering an inflammatory response. These inflammatory manifestations of obese AT have been linked to insulin resistance, metabolic syndrome, and type 2 diabetes, and proposed to evoke obesity-induced comorbidities including cardiovascular diseases (CVDs). A growing body of evidence suggests that metabolic disorders, characterized by AT inflammation and accumulation around organs may eventually induce organ dysfunction through a direct local mechanism. Interestingly, perirenal adipose tissue (PRAT), surrounding the kidney, influences renal function and metabolism. In this regard, PRAT emerged as an independent risk factor for chronic kidney disease (CKD) and is even correlated with CVD. Here, we review the available evidence on the impact of PRAT alteration in different metabolic states on the renal and cardiovascular function. We present a broad overview of novel insights linking cardiovascular derangements and CKD with a focus on metabolic disorders affecting PRAT. We also argue that the confluence among these pathways may open several perspectives for future pharmacological therapies against CKD and CVD possibly by modulating PRAT immunometabolism.

Keywords: perirenal adipose tissue, chronic kidney disease, cardiovascular disease, metabolic dysfunction, adipose tissue inflammation

INTRODUCTION

Adipose tissue (AT) is an active cellular complex that includes three different cellular types: white, brown, and beige adipocytes where extensive molecular, physiological and metabolic heterogeneity among different adipose depots exists (1–3). The main characteristic of white adipose tissue (WAT) is the large unilocular lipid droplet occupying most of the adipocyte volume. WAT functions as an excess lipid store in the form of triglycerides and secretes free fatty acids (FFA) to fulfill metabolic demands. Importantly, WAT regulates metabolic homeostasis by the synthesis and secretion of adipokines (4). Brown adipose tissue (BAT) is characterized by dispersed pockets of multilocular adipocytes and is rich in mitochondria. The main function of BAT is to dissipate energy through uncoupled respiration, that is mainly mediated by uncoupling protein-1 (UCP-1) (5). All of these AT depots are well vascularized, innervated by nerve structures, and contain preadipocytes, pericytes and immune cells (6). Recently, extensive research has uncovered the crucial role of AT depots. Not only does the physiological function of AT involve the maintenance of local and general homeostasis, *via* endocrine and paracrine activity, but also AT may contribute to the pathogenesis of many diseases (7–9). In this respect, the involvement of several fat depots was identified; perivascular adipose tissue (PVAT) is involved in the pathogenesis of hypertension (10) and epicardial AT is associated with atherosclerosis and coronary diseases (11).

Perirenal AT (PRAT) is yet another metabolically active AT depot. PRAT harbors an endocrine and paracrine role synthesizing and secreting adipokines pertinent to glucose and lipid homeostasis as well as inflammation (12). Interestingly, evidence shows that PRAT may influence the function and metabolism of the renal and cardiovascular system. Here, we summarize the recent findings regarding PRAT origin, structure and anatomical characteristics. We elaborate on the involvement of PRAT in different pathological conditions presenting new insights linking cardiovascular and renal diseases with a focus on metabolic disorders. An argument that the confluence exists among the pathways controlling metabolism and inflammation is made. This knowledge may represent a keystone for future novel approaches in metabolic, renal, and cardiovascular therapy and may open several perspectives in the field of PRAT immunometabolism.

PERIRENAL ADIPOSE TISSUE: ANATOMY, HISTOLOGY, AND ORIGINS

PRAT, a fat depot in the retroperitoneal space surrounding the kidney, was previously believed to act as mechanical support to the kidneys (13). However, recent studies highlighted that not only PRAT has an essential role in regulating kidney function but is also involved with cardiovascular system control. Anatomical studies have confirmed that PRAT exhibits an extensive blood supply, lymphatic channels, and neuronal innervation (14–16). Due to its interaction with renal blood vessels and possible exertion of physical hemodynamic effect, PRAT is believed to modulate the renal context in a manner analogous to that of PVAT in controlling blood pressure (17, 18).

The arterial blood supply to PRAT is derived from branches of the left colic, lower adrenal, renal, lumbar and ovarian/testicular arteries. This generates an abundant anastomosing capillary network supplying PRAT with oxygen and nutrients (19). Thus, PRAT is very well vascularized and is richly innervated (15, 20).

Although studies on the origin of PRAT are limited, emerging transcriptomic data provide insights into the unique nature of PRAT (2). Recent observations on PRAT adipogenesis revealed that preadipocytes are negative for endothelial markers (21). Indeed, human PRAT has been demonstrated to be a hybrid visceral AT, analogous to subcutaneous AT, and distinct from other visceral depots (2). Nevertheless, PRAT exhibits age-dependent molecular and morphological alterations. In human embryos, PRAT-derived adipocyte progenitors differentiated *in vitro* exhibit similar features of BAT including PRDM16 and UCP1 expression, as well as a comparable mitochondrion copy number, gene expression patterns, and oxygen consumption rates (22). In newborns, PRAT predominantly consists of brown adipocytes with a thin layer of WAT, which exhibit an age-dependent, progressive regression, such that adult PRAT appears to be predominantly white with dispersed pockets of multilocular adipocytes (23, 24). However, recent studies have shown that adult PRAT comprises spatially-distinct populations of dormant unilocular and multilocular UCP1-expressing adipocytes (21, 24–26). While unilocular UCP1-expressing adipocytes are evenly distributed within PRAT, multilocular UCP1-expressing adipocytes are located around the adrenal gland, in areas containing a higher number of sympathetic nerve endings (21). These two types of AT are associated with preadipocytes, mesenchymal stem cells, and several inflammatory cells (12). PRAT arises as a focal point in regenerative medicine, it is considered a depot for mesenchymal stem cells which manifest the ability to differentiate into adipocyte, osteogenic, chondrogenic and epithelial lineage (27). BAT progenitor cells are present in PRAT regardless of specific location (21). About 30% of PRAT population expresses UCP-1, the majority being multilocular and about 20% of them exhibiting a unilocular phenotype (21, 25).

The variability in PRAT morphology is also gender-dependent, PRAT is much more developed in males than females (28). Computed tomography measurements of PRAT were carried out in 123 individuals where males had higher PRAT volume than females at a comparable waist circumference (28). Another study confirmed the gender variability in PRAT thickness and volume compared to waist circumference (29). Gender based discrepancies are also reflected in the histological pattern of PRAT. BAT in PRAT has higher expression levels of UCP-1 in females than males (30). In cold weather, PRAT can show higher levels of BAT (25). The increase in browning capacity after cold exposure in females can be observed as heat is rapidly dispersed throughout the body and is attributed to the abundance of renal blood supply. Moreover, stronger browning capacity in females is associated with specific characteristics of

mesenchymal cells of PRAT and to a much lesser extent related to hormonal interventions (30). These findings are confirmed by a study on a murine model showing that Y-chromosome suppresses BAT UCP-1 expression (31).

When compared to other typical visceral AT, PRAT is more active in energy metabolism, synthesis, and secretion of several adipokines and inflammatory cytokines (12). PRAT manifests an immunoregulatory phenotype in response to several inflammatory cytokines as interleukin-1 beta (IL-1β), interferon (IFN), and tumor necrosis factor alpha (TNF-α) which could be targeted in anti-inflammatory therapy (27). These cytokines produced can regulate kidney function through paracrine or endocrine pathways. PRAT contributes to a decrease in kidney function in hypertensive individuals regardless of their body mass index (32). Furthermore, PRAT increases in prediabetic and diabetic patients and is associated with lower glomerular filtration rates in diabetic individuals (17, 18). This represents a potential immunomodulatory mechanism that could be targeted in different aspects of inflammatory conditions, tissue injuries (27), CVD and renal dysfunction.

PERIRENAL ADIPOSE TISSUE PHYSIOLOGY

Sympathetic Innervation

The autonomic nervous system is a key regulator of cardiovascular as well as energy homeostasis (33–35). The sympathovagal balance is essential in maintaining proper regulation of the cardiovascular and metabolic activity. Studies in human and experimental models indicate that sympathetic overflow induces hypertension (36, 37) and targeted end-organ damage (38, 39). Sympathetic nerve overactivity is detected in various tissues in obesity. Increased renal sympathetic nerve activity is reported in obese individuals and can be assessed by kidney norepinephrine spillover (40). Moreover, the sympathetic innervation regulates thermogenesis and energy liberation by innervating both the brown and white adipose pools (41–43).

The autonomic innervation into PRAT is functionally active (**Figure 1**). Indeed, the activation of afferent signals in the PRAT induces an increase in renal sympathetic activity (44). The afferent nerves in the adipocytes controlling the sympathetic outflow are referred to as an adipose afferent reflex (AAR) that modulates local homeostasis regulating energy balance and lipolysis (45–47). The activation of this sympatho-excitatory reflex in PRAT, AAR, can elevate sympathetic nerve activity and blood pressure (48, 49). The effect of the sympathetic innervation was even greater in hypertensive rat models or following a high fat diet (48, 49). Additionally, PRAT through AAR could regulate the sympathetic flow and therefore the cardiovascular system (50). Nevertheless, the function of the primary afferent neurons innervating PRAT remains unclear, further studies are essential to clarify the constituents of this pathway and the possible pathogenesis involved.

Given that PRAT thermogenic activation is essentially downstream of adrenergic stimulation, the inhibition of the sympathetic nervous system in obese rats by carotid baroreceptor stimulation not only alleviated metabolic derangements and insulin resistance, but also reduced PRAT mass and adipocyte hypertrophy, among other depots, by modulating the AMPK/PPARα/γ pathway (51). This was associated with a reduction of plasma and PRAT norepinephrine levels and an augmentation of acetylcholine levels (51). Moreover, rebalancing the autonomic nervous system by irradiating carotid baroreceptors in obese rats ameliorated metabolic dysfunction (52). This was associated with a restoration of norepinephrine and acetylcholine levels in PRAT, among other adipose depots, which rectified the AMPK/PPARα/γ pathway originally altered in obese rats (52). Although both interventions altered UCP-1 expression in BAT, neither altered UCP-1 expression in PRAT (51, 52). Nevertheless, it was demonstrated that systemic catecholamine excess in paraganglioma patients enhanced the prevalence of activated brown adipocytes in PRAT (53). Additionally, it was shown that high-fat diets as well as low protein, high carbohydrate diets induced PRAT browning that is associated with an upregulation of UCP-1, PRDM16, as well as β-AR expression (9, 54). Moreover, β_3-AR activation in HFD-fed obese rats not only enhanced FFA influx into PRAT, but also its utilization, observations similar to those in rats fed a low protein, high carbohydrate diet (54, 55). As PRAT represents a heterogenous and unique adipose depot, contrasting data emanating from these studies must be interpreted cautiously, and depending on the context. Nevertheless, the function of the primary afferent neurons innervating PRAT remains unclear, further studies are essential to clarify the constituents of this pathway and the possible pathogenesis involved.

Humoral Regulation-Adipokines

Adipokines encompass a group of endocrine proteins synthesized and released by adipose tissues functioning as regulators of the immune system and metabolism including insulin sensitivity and energy balance (56). These properties of adipokines are linked to metabolic dysfunction, CVD, and type 2 diabetes mellitus pathogenesis (57, 58). PRAT is highly active in adipokine synthesis as well as several pro-inflammatory cytokines (12, 59). PRAT secreted adipokines could affect the function of adjacent organs including the kidneys. Moreover, adipokines released into the systemic circulation could regulate CVS function (60, 61).

Leptin, an adipocyte-derived hormone, is a major regulator of hunger, energy homeostasis, and endocrine function (62). Circulating leptin increases in obesity through Janus kinase signal transducer and activator of transcription (JAK-STAT) pathway (63). Hyperleptinemia exacerbates atrial fibrosis and atrial fibrillation, as well as impaired glucose intolerance in obese mice (64, 65). Moreover, hyperleptinemia is associated with hypertension in obese men (66). Leptin injection into PRAT in rats stimulated the AAR without affecting the systemic sympathetic activity, highlighting that PRAT could directly regulate cardiovascular function (50). Moreover, in a rat model of metabolic syndrome (MetS), PRAT-derived leptin exacerbates the proliferation of glomerular endothelial cells by activating the MAPK pathway (67). Increased leptin synthesis in PRAT induced higher leptin concentrations in kidneys increasing the proliferation of glomerular endothelial cells through a cross-talk between the renin-angiotensin system (RAS) and leptin pathway, an effect that was reversed following the blockade of either RAS or leptin pathways (67). Therefore, aside from its systemic role,

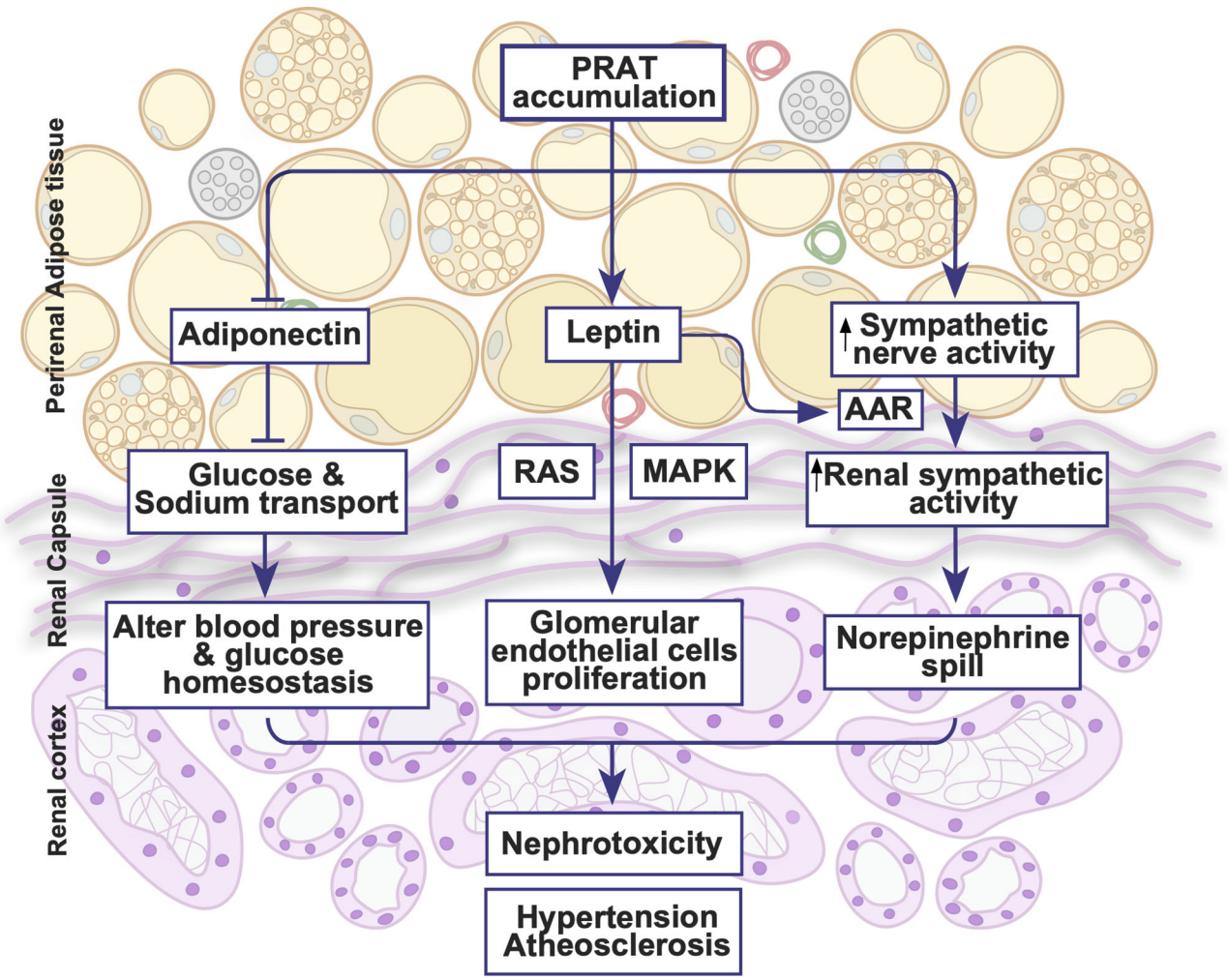

FIGURE 1 | Illustrative summary of perirenal adipose tissue physiology. PRAT exhibits an extensive blood supply, lymphatic channels and neuronal innervations. PRAT accumulation activates sympathetic activity, leptin secretion and renin angiotensin system all of which could lead to hypertension, atherosclerosis and nephrotoxicity. AAR, adipose afferent reflex; MAPK, mitogen-activated protein kinase; RAS, renin angiotensin system.

PRAT-derived leptin could directly affect endothelial cells and regulate RAS thereby affecting blood pressure as well.

Adiponectin is the most abundant adipokine in the human serum showing unique insulin-sensitizing, anti-inflammatory, cardioprotective, and antiapoptotic actions (60, 68). Adiponectin modulates metabolism; low levels of circulating adiponectin are linked to type 2 diabetes, atherosclerosis, and CVDs (69–71). Moreover, the activation of peroxisome proliferator-activated receptor delta (PPARδ) increases adiponectin secretion from PRAT, which exerts a protective effect on the renal tubular epithelial cells (72). The high salt diet-induced PPARδ activity inhibits sodium-glucose cotrasporter-2 (SGLT2) which promotes natriuresis and glycosuria. In diabetic states, patients show reduced natriuresis mainly due to impaired SGLT2 function. Moreover, reduced natriuresis in patients with uncontrolled hyperglycemia is correlated with low adiponectin levels. As a result, a distinctive role of adiponectin is revealed in regulating sodium and glucose homeostasis *via* SGLT2 in kidney tubules, a mechanism that is found to be impaired in diabetes (72).

Renin-Angiotensin System

The renin-angiotensin system (RAS) is a crucial regulator of energy metabolism, having a major role in several metabolic disorders including obesity and insulin resistance (73). RAS modulates adipocyte function, glucose, and triglyceride metabolism as well as lipolysis (74, 75). The expression of all components of the RAS, including angiotensin II (Ang II) and angiotensin 1-7 (Ang 1-7) and their receptors, have been recognized in adipocytes implying the involvement of local RAS in regulating AT function (74, 76). Recent studies have revealed the counteractive role of Ang II and Ang1-7 in regulating various functions of adipocytes (77).

Divergent findings have been reported assessing the regulation of RAS in AT depending on the type of adipose pool and different models studied [reviewed in (78)]. In rodents, the local

angiotensinogen synthesis in AT is increased following increases in food intake. In this model, angiotensinogen induced local Ang II synthesis, promoting AT growth (79). Moreover, in mice, the accumulation of AT is correlated with higher blood pressure an effect that is thought to be mediated *via* an increase in Ang II secretion from AT (80). In humans, Ang II has antiadipogenic effects in preadipocytes (81, 82). On the other hand, in obese hypertensive individuals, Ang II is increased highlighting a link between RAS and insulin resistance (83). In line, other clinical studies reported an increase in RAS components in obese individuals (84–87). Indeed, the local genetic expression of RAS in adipocytes of obese individuals is elevated (78, 88–91).

Recent studies showed that during adipogenesis, both Ang II production and Ang II type 1 receptor (AT_1R) are upregulated (92). The stimulation of AT_1R promotes leptin secretion in human adipocytes, an effect mediated *via* extracellular-signal-regulated kinases 1 and 2-dependent (Erk1/2) pathway (93). The stimulation of AT_1R in AT induces the production of several pro-inflammatory cytokines (94), which in turn stimulate the apoptosis of BAT and inhibit the browning of WAT (95). However, the blockade of AT1R curbs lipid accumulation and reactive oxygen species (ROS) generation in adipocytes. This was associated with increases in adiponectin and apelin and a decrease in the TNF-α, renin, and visfatin (92, 96).

On the other hand, Ang 1-7 pathway opposes the Ang II-AT_1R signaling, as it stimulates lipolysis and glucose uptake in the adipose pools and suppresses oxidative stress (97, 98). The activation of angiotensin-converting enzyme 2 (ACE2) *in vivo* reduces AT deposition (99). Ang 1-7 administration to rats on a high-fat diet was found to increase ACE2 expression and reduce AT accumulation (100). More studies on rats verified the previous results, in which Ang1-7 lipolytic effects were reduced *via* blockade of Mas receptors with a PI3K inhibitor (97). Nevertheless, blockers of AT_1R and AT_2R *in vitro* did not provoke changes in the Ang1-7 function.

PRAT fat deposition is presumed to activate RAS through compression of blood vessels, lymphatic system, and ureters, which leads to the development of hypertension, atherosclerosis and kidney dysfunction (101, 102). Low concentrations of Ang II increase adipocyte differentiation of human preadipocytes isolated from PRAT (103). Moreover, in states of metabolic dysfunction inflammatory cytokines derived from PRAT could be involved in nephrotoxicity (9, 104). The increase in PRAT inflammation in diabetic mice was reduced following Ang1-7 treatment. Additionally, Ang1-7 counteracted ROS production in PRAT (104). The functional significance of PRAT production of RAS components is an area of intense investigation, it could reveal a link between metabolic dysfunction, CVD, and CKD.

PERIRENAL ADIPOSE TISSUE INFLAMMATION AND METABOLIC COMPLICATIONS

Mechanisms Governing Adipose Tissue Inflammation and Thermogenesis

Metabolic homeostasis is governed by balanced, intricate, and opposing processes promoting energy acquisition and energy expenditure in order to maintain basal metabolic rates (105). An imbalance of such processes, exemplified by an increased energy acquisition due to caloric excess, is thought to drive early metabolic dysfunction, eventually culminating in the emergence of insulin resistance and its accompanying derangements such as obesity, type 2 diabetes, and CVD (106). Indeed, excessive caloric intake induces hyperinsulinemia, which drives adipocyte hypertrophy, promoting the diametric expansion of the AT beyond the diffusion potential of oxygen (107–109). WAT exhibits a decreased blood supply during hypertrophic remodeling resulting in a local hypoxic state. This is accompanied by an increased adipocyte oxygen consumption that is not made up for by proper compensatory vascularization, which triggers hypoxia-inducible factor-1 alpha (HIF-1α) expression and causes adipocyte death and subsequent inflammation (108). Indeed, hypoxia induces the release of proinflammatory cytokines, chemokines and angiogenic and fibrotic factors from adipocytes, favoring AT dysfunction and immune cell infiltration (61). Hypoxia-triggered expression of HIF-1α induces NF-κB-mediated cytokine production including IL-1β, which signals for the recruitment of circulating immune cells, causing an imbalance between homeostatic AT-resident immune cells and infiltrating proinflammatory immune cells (61, 110). Therefore, obesity is considered a state of chronic low-grade inflammation, in which infiltrating immune cells contribute to the hypoxic phenotype and to insulin resistance (111, 112). Additionally, hypoxia-induced AT dysfunction is associated with an extensive lipolytic activity and free fatty acids (FFA) release, promoting endoplasmic reticulum stress and adipocyte apoptosis (113, 114). In response to adipocyte death, the AT initiates a self-limiting reparative response by which infiltrating macrophages encircle apoptotic adipocytes creating histologically-distinguishable crown-like structures (61, 115). These macrophages aberrantly generate toxic ROS and nitric oxide (NO), which further damage neighboring cells, promoting tissue fibrosis (61). As the injurious signal persists, the chronic stimulation of myofibroblasts and immune cells exacerbates tissue damage, eventually leading to extracellular matrix remodeling, tissue fibrosis, and AT dysfunction (116).

There exists extensive heterogeneity among different adipose depots and among the adipocytes of a given depot itself, resulting in differential, depot-specific susceptibilities to inflammation (1–3). A long standing subcategorization of adipose depots differentiates between WAT and BAT. WAT comprises unilocular adipocytes that specialize in energy storage and adipokine secretion while BAT comprises mitochondria-rich, multilocular adipocytes that specialize in energy dissipation through non-shivering thermogenesis (5). Non-shivering thermogenesis encompasses intricate thermogenic pathways that are thought to occur downstream of β_3-adrenergic receptors (β_3-ARs), and in response to stimuli that enhance local sympathetic discharge including cold exposure and high fat diet consumption (5, 117, 118). These latter stimuli promote WAT browning, a phenomenon by which white adipocytes gain thermogenic potential, transforming into brown-like beige adipocytes. Emerging evidence implicates different thermogenic pathways downstream of β_3-ARs, that drive the thermogenic

potential of brown and beige adipocytes. The most efficient and quantitatively significant thermogenic effector is the inner mitochondrial membrane protein, UCP-1. UCP-1 is a fatty acid/H^+ symporter that uncouples mitochondrial oxidative phosphorylation from the production of ATP (5). Moreover, the activation of β_3-ARs induces lipolysis, increasing the levels of FFA, which further enhances UCP-1 activity. Nevertheless, it was shown that UCP-1 is dispensable for cold-induced and diet-induced thermogenesis (119). It was therefore hypothesized that less-efficient thermogenic pathways contribute to adaptive thermogenesis, the most prominent of which is creatine futile cycling (120). Creatine futile cycling, that is the phosphorylation of creatine by creatine kinase B and its subsequent futile hydrolysis, appears to take place in UCP-1-positive and UCP1-negative adipocytes (121, 122). Importantly, blocking creatine cycling in adipocytes either by impairing its endogenous biosynthesis or its transport, promotes diet-induced obesity and cold-intolerance in mice (123–125). Alternative thermogenic pathways also include lipolysis/re-esterification cycling that mediates adaptive thermogenesis based on the ATP demand of triacylglycerol synthesis, calcium cycling that is mediated by the SR/ER calcium ATPase pump and phospholamban, and the UCP1-independent proton leak by the mitochondrial ADP/ATP carrier that is initiated at high membrane potentials (5). Importantly, UCP1-dependent and UCP1-independent uncoupling of mitochondrial respiration from ATP production is linked to aberrant increases in the mitochondrial oxygen consumption rates and oxygen demand, thus possibly exacerbating AT hypoxia in states of metabolic dysfunction (7–9, 126–128).

Mechanisms Linking Perirenal Adipose Tissue Thermogenesis and Inflammation

Several studies have shown that an augmentation of central adiposity in overweight and obese individuals is associated with an increased PRAT mass, and that PRAT mass independently associates with insulin resistance and lower HDL-cholesterol levels (129). Moreover, PRAT thickness is associated with cardiovascular risk factors in a sex-dependent manner, where significant associations exist between increased PRAT thickness and fasting plasma glucose level, metabolic syndrome, and waist circumference in men, and only fasting plasma glucose level in women (130). Nevertheless, such observations cannot be merely explained by an increased PRAT mass. Recent mechanistic investigations possibly link PRAT thermogenesis and inflammation to renal dysfunction early in the course of metabolic disease (9). Although it could be inferred from clinical observations linking reduced PRAT browning to hypertension and metabolic dysfunction that PRAT browning holds therapeutic benefits, recent evidence in non-obese, prediabetic rats, links PRAT thermogenesis to localized inflammation, impairing renovascular function early in the course of metabolic disease (9, 131).

Indeed, increased UCP1 expression is consistently reported in different animal models of diet-induced obesity, a phenotype that is enhanced by the increased abundance of long chain fatty acids pertinent to these models (132, 133). Such an increased expression level of UCP1 drives diet-induced thermogenesis and is associated with increased levels of oxygen consumption (134). This is of particular relevance to adipose depots with inherently low expression levels of UCP1, such as the PRAT, in which diet-induced thermogenesis produces profound bioenergetic and inflammatory alterations (9). Indeed, PRAT inflammation and enhanced oxidative stress in prediabetic rats were associated with elevated glomerular filtration rate (GFR) accompanied by mild proteinuria in the absence of hypertension, hyperglycemia, obesity, and systemic inflammation (9). These rats exhibited an acutely increased production of ROS in PRAT which is suggested to have enhanced UCP-1 activity and mitochondrial respiration uncoupling (9, 135). Importantly, PRAT of HFD-fed rats exhibited an enhanced expression of UCP-1 which is thought to exacerbate local hypoxia and increase HIF-1α expression in the hypertrophied tissue by driving the aberrant consumption of oxygen. While studies of the PRAT UCP1 expression profile are scarce, the available evidence shows alteration of UCP1 expression in PRAT adipocytes in disease conditions such as hypertension and renal cell carcinoma (131, 136). In this context, mechanistic parallels can be drawn to PVAT, an intrinsically hybrid tissue harboring brown adipocytes, in which an increased UCP-1 expression exacerbates local hypoxia leading to AT dysfunction and inflammation and subsequent vascular derangements (8, 137). It was therefore suggested that UCP-1 may serve as a therapeutic target in select adipose depots to mitigate cardiovascular and renovascular derangements associated with early phases of metabolic dysfunction (61, 137). This is of particular relevance as the upregulation of PRAT UCP-1 expression and the excessive uncoupling of mitochondrial respiration not only deteriorated kidney function but were also associated with altered expression of epithelial and mesenchymal markers supportive of renal epithelial to mesenchymal transition (9).

Additionally, it was shown that PRAT altered adipokine profile and enhanced oxidative stress, inflammation, and fibrosis may partly explain the high risk of cardiovascular events observed in patients with primary aldosteronism or hypercortisolism (138, 139). In patients with aldosterone-producing and cortisol-producing adenomas, PRAT expressed significantly higher levels of IL-6 and TNF-α as well as fibrotic markers in comparison to normotensive individuals and patients with essential hypertension (138, 139). These observations are supported by *in vitro* experiments showing that aldosterone treatment of isolated human PRAT stromovascular cells, mouse 3T3-L1, and brown preadipocytes induces the expression of IL-6 and markers of inflammation and fibrosis (138). Additionally, the expression level of NADPH oxidase 4 (NOX4) significantly increased, while that of hemoxygenase-1 (HO-1) and nuclear factor erythroid 2-related factor 2 (Nrf2) significantly decreased in PRAT of patients with cortisol-producing adenoma (139). Indeed, dexamethasone treatment of pre-differentiated stromovascular cells, mouse 3T3-L1, and brown preadipocytes induces marked fibrosis and adipogenesis (139). Moreover, it was shown that dexamethasone treatment in adrenalectomized rats promotes hyperplastic PRAT expansion that is associated with an increased expression and dehydrogenase activity of 11 β-hydroxysteroid dehydrogenase type 1, an NADPH-dependent cortisone reductase (140). Moreover, hypercortisolism in patients with active Cushing's syndrome induced PRAT adipocyte hypertrophy, that was associated

with an increased macrophage infiltration, and augmented leptin and reduced adiponectin levels (141). Additionally, PRAT brown adipocytes were shown to be active in states of secondary hyperaldosteronism, such as in patients with pheochromocytoma (142). Nevertheless, the inflammatory landscape of PRAT in these patients was not assessed. However, it could be inferred from these studies that states of hyperaldosteronism and hypercortisolism drive both PRAT inflammation and increased UCP-1 indicating that possible cross-talks between both mechanisms plausibly exist.

PERIRENAL ADIPOSE TISSUE AND RENAL DISEASES

PRAT and Chronic Kidney Disease: A Possible Correlation

The strong correlation between body mass index and higher risk of chronic kidney disease (CKD) was first reported in 1974 and was found to be greatly associated with an increase in proteinuria (143). Patients with a high body mass index had a higher risk of CKD compared to lean individuals independent of age (144). Moreover, urinary albumin excretion was also elevated in obese patients who are neither hypertensive nor diabetic (145). Indeed, CKD is an independent complication of obesity; a metabolic profile identified as obesity-related glomerulopathy or obesity-related kidney disease (146, 147). Several studies highlighted the association between increased visceral adiposity, and particularly increased PRAT mass and volume, and determinants of metabolic and CV disease as well as kidney dysfunction (129, 148). Excess PRAT has also been associated with metabolic anomalies including insulin resistance, abnormal fasting blood glucose levels, hypertriglycemia, and higher uric acid excretion in urine in patients with CKD (129, 148).

Given the intimate relation between PRAT and the kidney, either due to their spatial proximity or due to their common innervation and vascularization, PRAT expansion, dysfunction, and inflammation are thought to have a pronounced impact on renal function (149, 150). PRAT thickness is increased in patients with MetS, which is accompanied by increases in oxidative stress and renal microvascular proliferation (151). PRAT expansion secondary to obesity contributes to kidney dysfunction irrespective of obesity (12, 32). This is in line with the observation that abdominal obesity was strongly associated with CKD compared to overall obesity (152, 153), where an increasing body of evidence has suggested that PRAT thickness is positively associated with visceral adiposity and waist circumference (145, 154). In this regard, excess PRAT was associated with a higher risk for CKD and could be used as a predictor for reduced GFR (145, 155, 156) and higher incidence of proteinuria in obese/overweight individuals (144, 157). A positive correlation was consistently found between PRAT thickness and microalbuminuria in obese patients (145, 156). Importantly, excessive PRAT inflammation is believed to exacerbate renal vascular and endothelial damage (12, 59, 158, 159). Moreover, it was recently shown that PRAT exhibits an age-dependent inflammatory signature that is characterized by an increased peri-organ recruitment of macrophages and inflammatory natural killer (NK) cells in the vascular stromal fraction that was associated with a deleterious impact on the microenvironment of renal transplants (160, 161). Nevertheless, another study failed to arrive at such association, where PRAT inflammation did not correlate with the reduced early renal graft function observed in obese kidney donors (162). Based on the previous findings, excess PRAT could be considered an independent predictor of CKD. Additionally, the ultrasound evaluation of PRAT is now proposed as a parameter that might be useful for early assessment of obesity-induced renal dysfunction (145).

Possible Mechanisms

The detailed mechanisms by which PRAT initiates and exacerbates chronic renal injury have not been completely elucidated, but several pathways are postulated (**Figure 2** and **Table 1**). First, the accumulation of PRAT on the kidney may result in a direct obstruction of renal parenchyma and vessels. This might induce increases in sodium reabsorption and higher blood pressure as a consequence in addition to alterations in kidney function in obese patients (24). The firm encapsulation of the kidney could further increase the interstitial hydrostatic pressure and reduce renal blood flow (149, 167). Consequently, the increase is sodium absorption leads to a decreased sodium chloride delivery to the macula densa resulting in lower resistance in afferent arterioles which leads to increased GFR and altered RAS (167–170). All of these processes including an increase in interstitial hydrostatic pressure, stimulation of renin release, glomerular filtration and sodium tubular reabsorption accelerate renal disease progression eventually leading to GFR reduction (24, 148, 149). Recently, a study on hypertensive individuals has reported a decline in GFR that was correlated to an increase in PRAT specifically among other visceral AT depot regardless of gender (32). As a consequence of GFR reduction, high uric acid and triglycerides were correlated with PRAT thickness in patients with CKD (24, 148).

Second, chronic inflammation due to the increase in FFA production is the hallmark of obesity and therefore can also be associated with modulation of PRAT function. Indeed, the increased volume of PRAT is positively associated with overproduction of FFA (145). Metabolites of FFA have a direct renal lipotoxic effect and are directly correlated with albuminuria (145, 171). Levels of FFA in renal venous blood were significantly higher than those in the jugular vein, this indicates the involvement of PRAT in kidney damage through direct intercellular signaling pathways (163). The excessive release of FFA by PRAT could directly impair endothelial function by enhancing the oxidation of tetrahydrobiopterin and uncoupling of endothelial nitric oxide (NO) synthase, leading to the production of L-arginine or superoxide instead of NO (172). The reduced NO synthesis could lead to a compensatory mechanism synthesizing vascular endothelial growth factor, leading to a greater albumin leak from the glomerulus (172). Furthermore, FFA-induced renal lipotoxicity could exacerbate chronic inflammation by increasing the metabolism of intracellular fatty acids (171).

Third, excess PRAT can affect renal function through a local or systemic secretion of pro-inflammatory mediators which may

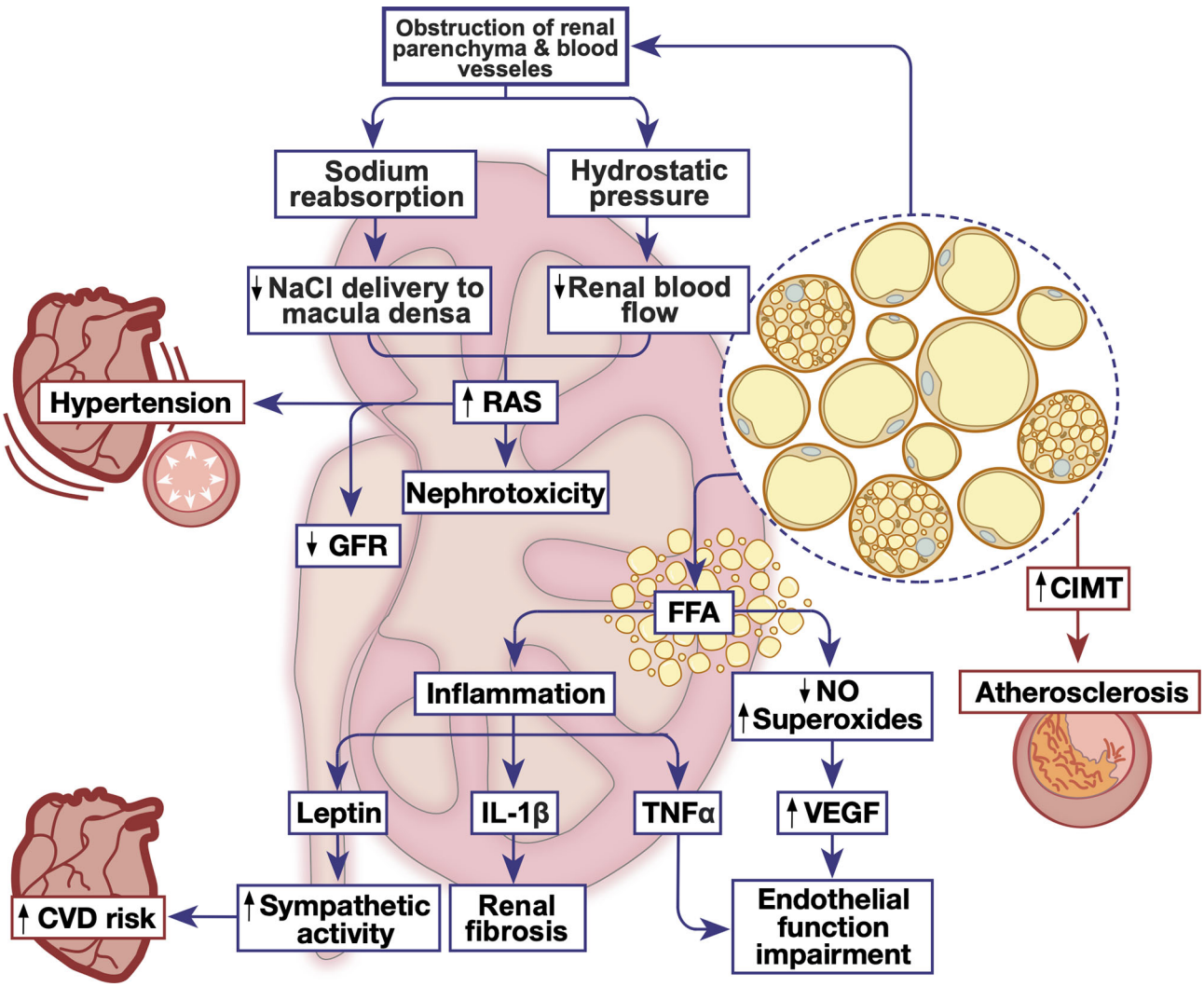

FIGURE 2 | The emerging role of PRAT in renal and cardiovascular homeostatic function. Metabolic dysfunction triggers PRAT deposition and inflammation leading to alterations in cardiovascular and renal function, triggering nephrotoxicity and cardiovascular diseases. CIMT, carotid intima-media thickness; CVD, cardiovascular disease; FFA, free fatty acid; GFR, glomerular filtration rate; IL-1β, interleukin-1 beta; NO, nitric oxide; RAS, renin angiotensin system; TNFα, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

influence the kidney function in a paracrine manner (12, 21, 170, 173). In obese swine, excess PRAT secretes tumor necrosis factor-α (TNF- α) which impairs renal endothelial function (158). This could be also related to the loss of NO as discussed previously. Moreover, the expression of plasminogen activator inhibitor-1 (PAI-1) in PRAT was increased in high-fat diet-induced obese mouse model (165). PAI-1 is shown to contribute to macrophage mediated inflammation, extracellular matrix accumulation and thus diabetic nephropathy. The genetic deletion or the inhibition of PAI-1 reduced PRAT inflammation, renal histological lesions and abated renal fibrosis (165). Excessive PRAT secretion of TNF- α and leptin could trigger renal fibrosis in murine models (29, 174, 175). Leptin exacerbates vascular remodeling and glomerular proliferation *via* activation of the p38 MAPK pathway (67). Concomitantly, TNF- α induces direct renal endothelial dysfunction thus modulating GFR (67, 158). Leptin secretion could also stimulate the sympathetic nervous activity *via* altering the proopiomelanocortin-melanocortin 4 receptor pathway in the central nervous system (160). Interestingly, our recent study on non-obese prediabetic rats has shown that a localized PRAT inflammation, presenting as higher IL-1β expression, evoked renal structural and functional deterioration associated with an altered renovascular endothelial function (9). Importantly, a recent study reported a direct correlation between age and inflammatory profile of donor-derived stromal vascular fraction of PRAT (PRAT-SVF), expressed by a local recruitment of NK cells. These NK cells are associated with NKG2D receptor activation and encodes for INFγ, indicating that NK cells derived could be involved in the pro-inflammatory pathway leading to renal dysfunction in elderly patients with transplants (160).

TABLE 1 | Main findings of studies linking perirenal adipose tissue to renal disorders.

Main finding	Possible mechanism	Conducted on	Reference
PRAT is associated with increased urinary albumin excretion	Low adiponectin and elevated leptin levels trigger pathways augmenting renal inflammatory and oxidative stress leading to renal vascular dysfunction causing increased urinary albumin excretion	obese rats	(163)
PRAT promotes renal arterial endothelial dysfunction	Accumulation of PRAT showed increases in inflammation and oxidative stress, which triggered renal endothelial dysfunction *via* TNF-α acting in a paracrine manner.	Pigs with obesity and metabolic derangements	(158)
PRAT accumulation was correlated with a decline in GFR	–	Hypertensive patients	(32)
PRAT thickness was negatively correlated with GFR	–	Diabetic patients	(164)
PRAT-derived leptin has a detrimental effect on the kidney	PRAT hypertrophy induces an increase in leptin expression that is accompanied by an imbalance in the expression of the Ang II–AT_1R and ACE2–Ang(1–7)–Mas receptor axes. This promotes glomerular endothelial cells proliferation by activating p38 MAPK pathway.	Rats with metabolic syndrome	(67)
PRAT inflammation and macrophage infiltration are linked to high fat diet induced nephropathy	Expression of plasminogen activator inhibitor-1 (PAI-1) in PRAT was increased. PAI-1 contributes to macrophage mediated inflammation, extracellular matrix accumulation and thus diabetic nephropathy.	Obese mouse model	(165)
Localized PRAT inflammation evoked renal impairment in early course of metabolic deterioration	The paracrine effects of PRAT inflammation, presented as higher IL-1β expression, lead to renovascular endothelial dysfunction, hyperfiltration, renal cortical inflammation and proteinuria.	Non-obese prediabetic rats	(9)
PRAT Inflammation exacerbates diabetic nephropathy.	Ang1-7 supplementation to these mice not only reduced renal mesangial expansion and urinary albumin secretion, but also ameliorated renal fibrosis and PRAT oxidative stress and inflammation mainly through the attenuation of NOX-mediated ROS production.	*db/db* mice	(166)
Excessive perirenal adiposity may constitute an independent prognostic factor of kidney malfunction	Inducing the heme oxygenase system in diabetic fat rats reduced PRAT adiposity, macrophage infiltration, and the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6.	Zucker-diabetic fatty rats	(159)

Additionally, as both obesity, diabetic nephropathy, and primary hypertension are associated with an enhanced activity of the RAS, it becomes plausible that the enhanced activity of this system in PRAT and in the adrenal gland participates in the pathogenesis of obesity-associated hypertension and diabetic nephropathy, particularly through Ang II-mediated PRAT dysfunction (101, 102). The inhibition of ACE in a uninephrectomized rat model of chronic renal dysfunction prevented PRAT mass accumulation and led to the emergence of multilocular thermogenic adipocytes (176). Indeed, it was shown that Ang1-7 counteracts the detrimental effects of Ang II on diabetic nephropathy in db/db mice (166). Ang1-7 supplementation to these mice not only reduced renal mesangial expansion and urinary albumin secretion, but also renal fibrosis and PRAT oxidative stress and inflammation mainly through the attenuation of NOX-mediated ROS production (166). Nevertheless, it remains unclear whether Ang1-7 reduced renal dysfunction by virtue of its ameliorative effect on PRAT inflammatory and oxidative status. Moreover, such mechanisms contributing to diabetic nephropathy were shown in Zucker-diabetic fatty rats (159). Inducing the heme oxygenase system in these rats reduced PRAT adiposity, macrophage infiltration, and the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 (159). Moreover, the hemin-mediated enhancement of the heme oxygenase system in PRAT reduced the proinflammatory M1 macrophage polarization while inducing the anti-inflammatory M2 macrophage polarization. These effects were accompanied by reduced renal damage and fibrosis and enhanced kidney function. As these models exhibit extensive damage that is associated with diabetes, the dissection of a causality relationship between PRAT inflammation and renal dysfunction remains marginally possible. Another study in non-diabetic, high fat diet-fed rats highlighted PRAT association with increased urinary albumin excretion due to renal vascular dysfunction caused by the activation of renal inflammatory and oxidative stress augmenting pathways (163). Nevertheless, the inflammatory condition of PRAT was not assessed and thus these observations were attributed to increased visceral adiposity, and particularly increased PRAT mass. However, it was shown in non-obese prediabetic rats that subtle PRAT inflammation induces pronounced kidney dysfunction in isolation of hyperglycemia and systemic inflammation (9). It therefore becomes plausible that PRAT inflammation in the setting of metabolic dysfunction might precede renal manifestations of the disease.

PRAT thickness could aggravate renal anomalies due to metabolic dysfunction such as abnormal insulin serum levels, high glucose or triglycerides and uric acid, all of which are reported in patients with CKD (129). Additionally, excess PRAT is detected in patients with calcium phosphate apatite or uric acid nephrolithiasis (177). However, future research into analyzing the detailed mechanism is warranted.

RENAL DISEASES AND CARDIOVASCULAR DYSFUNCTION: CARDIORENAL SYNDROME

A strong relationship exists between the heart and kidney where the proper function of one depends on the other. Cardiorenal syndrome is defined as 'disorders of the heart and kidney whereby acute or chronic dysfunction in one organ may induce acute or chronic dysfunction of the other' ultimately leading to failure of

both organs (178). CKD is strongly associated with CVD, it has been reported that patients with advanced kidney disease are at high risk of CVD mortality and morbidity (179). Recent studies reported a 40-50% cardiovascular mortality rate in patients with advanced and end-stage CKD compared to 26% in patients with normal kidney function (180, 181). Nonetheless, the correction of the classical cardiovascular risk factors, including hypertension, diabetes, and dyslipidemia, in patients with CKD was not sufficient in neutralizing the impact of CKD on cardiovascular risk (182). It is also now being recognized that patients with early stages of CKD also suffer from a high risk of cardiovascular events (183) possibly implicating a role for PRAT inflammation in this observations. Consistent with these observations, CKD is now considered an independent risk factor for CVD (184). It is also worth mentioning that patients with CKD are more likely to die from CVD rather than terminal end-stage kidney disease (185).

PERIRENAL ADIPOSE TISSUE AND CARDIOVASCULAR DYSFUNCTION

Obesity and specifically visceral fat accumulation are actively involved in the pathogenesis of CVDs including hypertension (186) and coronary heart diseases (CHD) (187). Abdominal obesity has a close association with MetS and CVD (188). Excess visceral AT poses a high risk for dyslipidemia, hypertension and cardiorenal disorders (189). Studies have shown that risk of CVDs is more closely related to visceral fat volume, including PRAT, compared to subcutaneous AT (130, 148, 190). However, surgical lipectomy of abdominal AT depots was not effective in improving metabolic function or reducing CVD risk (191). Therefore, a need exists to identify other AT pools in which certain interventions could lower the risk of developing CVDs. Notably, recent findings have identified PRAT specifically as an emerging risk for CVD independent of metabolic profile (190) (**Figure 2** and **Table 2**).

Hypertension is a common manifestation of CVD and is even associated with a higher risk of all-cause mortality (198). Hypertension increases the risk of myocardial infarction, congestive heart diseases, and stroke (12). Proper control of blood pressure is fundamental in CVD treatment and prevention of further complications. There is a linear relationship between hypertension and obesity, around 60-76% of obese individuals have hypertension (199). Indeed, PRAT thickness is positively correlated with blood pressure in obese and overweight patients (190). In obese individuals, PRAT thickness is considered as an integral parameter defining both the risk for developing arterial hypertension and chronic renal disease (192). Twenty-four hours mean diastolic blood pressure was reported to be a dependent variable of PRAT and aldosterone production independent of metabolic parameters (190). Similar results were reported in patients with polycystic ovary syndrome, a positive correlation between PRAT thickness and blood pressure in patients was documented (193). Excessive PRAT accumulation compresses the kidney, increase RAS sodium reabsorption, and induce hypertension (149, 168). There is evidence that PRAT could directly regulate the cardiovascular system through an amplified afferent signals by the AAR, resulting in an increase in the renal sympathomimetic outflow (200). PRAT hypertrophy activates macrophage infiltration and proinflammatory cytokine release that adversely affect systemic vascular function (201). Altogether, these results suggest that PRAT has a direct role in controlling blood pressure, however; further studies are recommended to assess the detailed mechanistic pathway behind this phenomenon.

Additionally, atherosclerosis is a key factor in inducing CVDs and a leading cause of morbidity and mortality (202). Carotid intima-media thickness (CIMT) assessment is considered as a marker of subclinical atherosclerosis (203) and is therefore used to determine the risk of CVDs in clinical trials (204). Indices of atherosclerosis including body weight, waist circumference can predict CHD. Alterations in AT metabolism underlie atherosclerosis and thus CHD (205). More specifically, clinical studies reported that body fat distribution has a significant correlation with the severity of CHD without any clinical evidence of CVD. Compared to other fat depots, PRAT thickness had the highest partial correlation coefficient with CVD, highlighting the contribution of PRAT in early cardiovascular changes in males and females (12). Notably, excess PRAT is positively correlated with indices that predict atherosclerosis as CIMT, waist to height and waist to hip ratio, waist circumference, and abdominal height (194). A thickness level of 0.26 cm of PRAT, measured using abdominal ultrasound, was found to determine the presence of sub-clinical atherosclerosis (194). Moreover, in HIV-infected patients, having a high risk of CVD, similar results were obtained. Patients with HIV and visceral adiposity exhibited high

TABLE 2 | Main findings of studies linking perirenal adipose tissue to cardiovascular disorders.

Main finding	Targeted population and references
PRAT accumulation was correlated with higher blood pressure, which was also dependent on urinary concentrations of aldosterone independent of metabolic parameters.	Obese and overweight individuals (190)
A significant direct correlation between PRAT thickness and hypertension	Hypertensive and non-hypertensive obese individuals (192) Patients with polycystic ovary syndrome (193)
PRAT thickness has shown to be significantly correlated with indices that predict atherosclerosis.	Male and female subjects (194)
PRAT thickness was associated with atherosclerosis specifically intima-media thickness of the common carotid artery	Male subjects (195) HIV-infected patients (196)
PRAT was associated with diverse metabolic and cardiovascular risk factors including carotid intima-media thickness	Asymptomatic prepubertal Caucasian children (197)

PRAT thickness and intima-media thickness of the common carotid artery. This implies the association of PRAT thickness and atherosclerosis in these individuals (196). Importantly, the correlation between PRAT and CIMT was even detected in children (197). Being readily available, abdominal ultrasound can easily measure PRAT thickness and therefore a prompt initiation of action to mitigate or prevent atherosclerosis and control CVD can be achieved.

Considering the above-mentioned associations, excess PRAT contributes to alterations in vascular and metabolic functions associated with CVD. PRAT is also potentially related to epicardial fat, both having mesothelial layers enriched with WAT progenitors that produce adipocytes (129). The coexistence of metabolic dysfunction, hypertension, and inflammation impacts end organ function gradually initiating a cascade of events that exacerbate CVD making it more resistant to treatment. These findings confirm the suggested hypothesis that excess PRAT is a risk factor of CVDs and a predictor for cardiac dysfunction.

FUTURE PERSPECTIVE AND POSSIBLE TREATMENT

Proper understanding of the molecular alterations regulating AT dysfunction could identify therapeutic targets to promote healthy AT expansion, hence preserving renal and cardiovascular function. AT dysfunction is the central mechanism for the development of complications related to obesity and metabolic diseases; therefore, most of the therapeutic approaches to mitigate AT dysfunction are related to indices of obesity (206). Interventions targeting AT dysfunction rely on a foundation of lifestyle modification including low caloric intake, suitable exercise, and fasting (207). Pharmacological tools and surgeries could be implemented to help the patients reach their health goals (207). As the potential harmful effects of excessive PRAT accumulation in renal and cardiovascular diseases were outlined, targeting PRAT inflammation emerges as a potential approach to combat the complex mechanisms implicated in CKD and CVDs.

Bariatric surgery is an effective intervention in controlling type 2 diabetes, dyslipidemia and quality of life of morbidly obese patients (208). Ricci et al. (192) showed that bariatric surgery could significantly decrease PRAT as well as blood pressure in morbidly obese patients. Moreover, as previously mentioned, PRAT is capable of reactivating dormant BAT into active BAT by cold exposure or stimulation of β_3- adrenergic receptors. This property represents a promising strategy to combat AT inflammatory conditions leading to metabolic diseases (25, 55).

Possible pharmacological interventions include several drug classes. Beyond their lipid-lowering effects, statins, 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors, were found to have anti-inflammatory, anti-oxidant and anti-proliferative properties (209). Statins also increase adiponectin levels, protect the endothelium and reduce urinary albumin excretion rate in patients with diabetes (210, 211). Pravastatin treatment for 6 months in patients with documented coronary artery diseases significantly increase serum adiponectin concentration (212). Higher adiponectin levels following statin treatment improves endothelial function (213), inhibit vascular smooth muscle cell proliferation (214, 215), and modulate vascular inflammatory cascades (216, 217). Despite these findings, to date no data link statin treatment with changes in the PRAT depot, however one could speculate a beneficial role of statins in PRAT dysfunction.

Other pharmacological tools to modulate PRAT inflammation are anti-diabetic drugs namely, metformin and pioglitazone. A two-week treatment with non-hypoglycemic doses of either drug was shown to ameliorate PRAT inflammation in pre-diabetic rats on mild hypercaloric diet. Interestingly, treatment reversed the deterioration of renal function triggered by 12 weeks of a mild hypercaloric feeding (9). Interestingly, a recent study showed treatment with a sodium/glucose co-transporter 2 (SGLT2) inhibitor reduced urinary albumin excretion and glomerular cell proliferation in high-fat fed mice, which was accompanied with decreased PRAT inflammation and leptin production (218). Indeed, the impact of SGLT2 inhibitors on PRAT inflammation is proposed to proceed through the activation of AMPK/Sirt1 pathway and potential restoration of autophagy (219). Moreover, short-term treatment with glucagon-like peptide receptor agonists was shown to reduce PRAT thickness and improve renovascular function in diabetic patients (220). Specifically, liraglutide treatment reduced perirenal adipocyte size in diabetic mice (221). Nevertheless, identifying specific drugs to target PRAT accumulation or inflammation require further investigation and research.

CONCLUSION

This era is marked by major changes in the traditional perception of AT physiological and metabolic function. The proximity of PRAT to the kidney makes its specific anatomical and morphological features relevant to renal function and general homeostasis. In this context, regardless of the small size of PRAT compared to visceral or subcutaneous AT, the effect of the adipokines and cytokines secreted broadens the impact of PRAT in maintaining metabolic, renal, and cardiovascular homeostasis. The data concerning the unique nature and pathophysiology of PRAT is limited, but studies are underway to unveil the potential molecular factors involved in PRAT function opening promising perspectives in developing appropriate therapeutic and preventive approaches.

AUTHOR CONTRIBUTIONS

SH and IA participated in literature review and screening and contributed to manuscript writing. SH wrote the first draft of the manuscript. AE and YA helped inn overseeing and coordinating the work and participated in manuscript draft review. AE-Y developed the idea, supervised the work, reviewed and modified manuscript draft, and provided research funding support. All authors contributed to the article and approved the submitted version.

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REVIEW
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Role of Inflammation in Vascular Disease-Related Perivascular Adipose Tissue Dysfunction

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Perivascular adipose tissue (PVAT) is the connective tissue around most blood vessels throughout the body. It provides mechanical support and maintains vascular homeostasis in a paracrine/endocrine manner. Under physiological conditions, PVAT has anti-inflammatory effects, improves free fatty acid metabolism, and regulates vasodilation. In pathological conditions, PVAT is dysfunctional, secretes many anti-vasodilator factors, and participates in vascular inflammation through various cells and mediators; thus, it causes dysfunction involving vascular smooth muscle cells and endothelial cells. Inflammation is an important pathophysiological event in many vascular diseases, such as vascular aging, atherosclerosis, and hypertension. Therefore, the pro-inflammatory crosstalk between PVAT and blood vessels may comprise a novel therapeutic target for the prevention and treatment of vascular diseases. In this review, we summarize findings concerning PVAT function and inflammation in different pathophysiological backgrounds, focusing on the secretory functions of PVAT and the crosstalk between PVAT and vascular inflammation in terms of vascular aging, atherosclerosis, hypertension, diabetes mellitus, and other diseases. We also discuss anti-inflammatory treatment for potential vascular diseases involving PVAT.

Keywords: perivascular adipose tissue, vascular diseases, inflammation, endocrine, crosstalk

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INTRODUCTION

The vascular system is a highly branched network lined with endothelial cells (ECs) and vascular smooth muscle cells (VSMCs), which can provide oxygen and nutrition for tissues. The regulation of vascular function in response to changing metabolic needs is essential for the maintenance of normal tissue and organ functions; it is also important for health preservation and disease prevention (1). Vascular diseases caused by vascular injury and dysfunction are among the top five causes of death among non-communicable diseases worldwide; these diseases influence various other diseases, such as heart diseases, nervous system diseases, and metabolic disorders (2). Vascular homeostasis is regulated by many factors, among which perivascular adipose tissue (PVAT) plays an important role in the pathogenesis of vascular diseases.

PVAT, which surrounds most blood vessels (except cerebral vessels) (3), is a connective tissue composed of adipocytes, preadipocytes, mesenchymal stem cells, fibroblasts, inflammatory cells

(macrophages, lymphocytes, and eosinophils), vascular cells, and nerves; these cells form adipose tissue microvasculature (4). PVAT is characterized by a reduced degree of differentiation, compared with classical visceral fat (PVAT more closely resembles preadipocytes); moreover, it exhibits a tendency to release pro-inflammatory factors and growth factors (5). Because of the anatomical characteristics of its adjacent vessel walls, PVAT provides mechanical support in the vascular system, particularly during adjacent tissue contraction. Furthermore, PVAT releases various factors, including adipokines and cytokines/chemokines. Through paracrine/endocrine mechanisms, these factors can directly diffuse or reach the vascular endothelial layer through blood vessels or a network of small mediators that connects the middle layer and lower adventitia. Additionally, these factors regulate vascular tension, cell proliferation, and cell migration; exhibit considerable influence on vascular homeostasis and function; and demonstrate both protective and harmful effects on the vascular system, according to pathophysiological characteristics present in the tissue microenvironment.

Under physiological conditions, PVAT has anti-inflammatory effects, improves free fatty acid metabolism, and regulates vasodilation. However, in the event of vascular pathology, PVAT increases in volume and becomes dysfunctional. This leads to changes in cell composition and molecular characteristics, as well as extensive secretion of pro-inflammatory and anti-vasodilation factors; it also promotes the infiltration of inflammatory immune cells and local oxidative stress, triggering vascular wall "from the outside to the inside" pathological signal, thereby causing VSMC and EC dysfunction (6, 7). The specific mechanisms and characteristics of PVAT dysfunction may differ among vascular diseases, despite important similarities with respect to inflammation characteristics. Inflammation is also an important pathophysiological event in vascular aging, atherosclerosis, hypertension, diabetes mellitus (DM), and other vascular diseases (8, 9). Therefore, the pro-inflammatory crosstalk between PVAT and blood vessels may comprise a novel therapeutic target for the prevention and treatment of vascular diseases. In this review, we summarize the latest findings regarding PVAT function and inflammation in various pathophysiological contexts and discuss anti-inflammatory treatments for potential PVAT-related vascular diseases.

SECRETORY FUNCTIONS OF PVAT

PVAT is considered an important endocrine tissue for the maintenance of intravascular stability. Although most inflammation in PVAT is attributed to infiltration by macrophages and T cells, PVAT-related regulation of vascular function depends largely on its secretory functions (**Figure 1**). Dysfunctional adipocytes themselves exhibit pro-inflammatory phenotypes and may play important roles in triggering and spreading inflammation within PVAT (10). Similar to other adipose tissues, PVAT secretes many adipose tissue-specific adipokines, chemokines, and growth factors; these directly affect the functions of adjacent blood vessels in a paracrine manner and can also reach the lumens of adjacent blood vessels, then have various downstream effects. PVAT affects tension and endothelial functions throughout the vascular bed in a vascular secretion manner, thus triggering and coordinating the infiltration of inflammatory cells (e.g., T cells, macrophages, dendritic cells, B cells, and NK cells) (11).

Under physiological conditions, PVAT mainly secretes anti-inflammatory adipokines, such as adiponectin (APN), omentin, fibroblast growth factor-21 (FGF-21), and nitric oxide (NO) (**Figure 1A**). In the context of vascular dysfunction, PVAT mostly produces and releases pro-inflammatory adipokines, such as leptin, tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1, also known as CCL2), RANTES (Chemokine C-C motif ligand 5, CCL5), interleukin-6 (IL-6), and interleukin-1β (IL-1β); all of these factors can directly affect VSMCs and ECs, thus triggering and coordinating vascular inflammation (**Figure 1B**) (5, 9).

PVAT and Anti-Inflammatory Cytokines

Under physiological conditions, PVAT releases various anti-inflammatory factors, including APN. The main biological

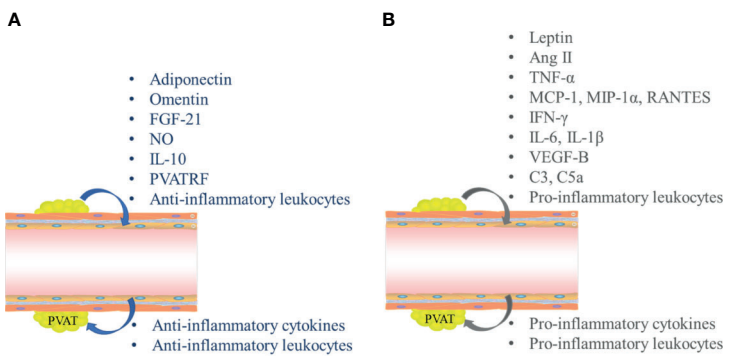

FIGURE 1 | Secretory functions mediate inflammatory crosstalk between perivascular adipose tissue (PVAT) and blood vessels. **(A)** Interactions between PVAT and blood vessels in normal physiological conditions. **(B)** Crosstalk between PVAT and blood vessels in pathological conditions. FGF-21, fibroblast growth factor-21; NO, nitric oxide; IL, interleukin; PVATRF, PVAT-derived relaxing factor; TGF-β1, transforming growth factor-β1; Ang II, angiotensin II; TNF-α, tumor necrosis factor-α; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein 1α; IFN-γ, interferon γ; VEGF-B, vascular endothelial growth factor B.

functions of APN include promotion of fatty acid biosynthesis and inhibition of gluconeogenesis in liver, enhancement of glucose uptake in skeletal muscle, improvement of systemic insulin resistance, and prevention of systemic atherosclerosis by increasing fatty acid oxidation (12).

The effects of APN-mediated anti-inflammatory action on vascular function have been elucidated in multiple vascular diseases. Compared with mice that were fed a regular chow diet, the anti-contractile effect of PVAT was significantly reduced in non-endothelial aortic rings in mice that were fed a high-fat diet, partly because of reduced APN release from PVAT, associated with AMPK dysfunction and/or PVAT inflammation (13–16). APN expression is reportedly downregulated in desoxycorticosterone acetate (DOCA)-salt hypertensive mice because of complement activation in PVAT; this effect can be reversed by macrophage depletion (17). Transplanted PVAT exhibits reduced expression of APN, thereby aggravating endothelial dysfunction through an inflammatory response (18). Exogenous APN can restore aortic anti-contractile activity of adult male offspring in mice exposed to gestational intermittent hypoxia (19). Upregulation of the heme oxygenase-1/APN axis in PVAT mediates anti-contractility-related Irisin improvement in the thoracic aorta of obese mice (15, 20). Chronic APN therapy can inhibit chemokine and pro-inflammatory adipokine expression patterns in PVAT in both aged rats and rats that were fed a high-fat diet, thus improving endothelial dysfunction in these models (21). PVAT around the coronary artery, collectively classified as epicardial adipose tissue (EAT), is closely related to the occurrence and development of coronary atherosclerotic lesions, as well as plaque stability. Angiotensin converting enzyme 2 (ACE2)-knockout mice that were fed a high-fat diet exhibited increased EAT inflammation; this was associated with decreased cardiac APN, decreased AMPK phosphorylation, increased cardiac steatosis and lipotoxicity, and myocardial insulin resistance, which exacerbated cardiac functional damage (22). In EAT from patients with coronary heart disease (CHD), the levels of APN decreased, while the levels of IL-6, TNF-α, and Toll-like receptor 4 increased. APN administration has been shown to prevent atherosclerosis by reducing the production of TNF-α in macrophages and reactive oxygen species (ROS) in endothelial cells; it also increases endothelial cell migration and angiogenesis (23). Samples from humans indicate that type 2 diabetes (T2DM) is closely related to hypoadiponectin, suggesting that the APN signaling pathway can serve as a new route for vascular protection in blood vessels and PVAT. However, the molecular mechanism by which APN reduces vascular inflammation remains unclear. Inhibition of nuclear factor kappa-B (NF-κB) signaling (which regulates several pro-inflammatory genes) and its transcription factors may be another mechanism by which APN alleviates vascular dysfunction (24).

Omentin (also known as intelectin-1, lactoferrin receptor, or endothelial lectin) has a positive effect on vascular inflammation. By inhibiting the thioredoxin-interacting protein/nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 signaling pathway, omentin can reduce the production of pro-inflammatory cytokines (e.g., TNF-α, IL-6, and IL-1β) and increase the production of anti-inflammatory cytokines (e.g., APN and IL-10) in obese mouse adipose tissue, as well as macrophages co-cultured with lipopolysaccharides (25, 26). The anti-contractile effect of PVAT in a physiological environment was lost in patients with T2DM, although it partially recovered after treatment with omentin-1, the main cyclic forms of omentin (27). Furthermore, omentin-1 treatment significantly improved the pro-inflammatory and pro-oxidant PVAT phenotype (i.e., through reduction of C-reactive protein and nitrotyrosine levels), suggesting that omentin-1 could improve endothelial dysfunction in T2DM patients by improving dysfunction PVAT; it also has the potential to treat T2DM-related vascular complications (28). In patients with atrial fibrillation and valvular heart diseases, the expression of omentin was downregulated in EAT and right atrial appendage tissue (29). Importantly, the expression of omentin in EAT was lower in patients with CHD than in patients without CHD; the expression of omentin in EAT was lower around stenotic segments of coronary artery than around non-stenotic segments (30). However, another study showed that, compared with the control group, the expression of omentin in EAT increased in patients with CHD, despite reduction of its circulating level; this finding suggested that omentin may play a local role in the development of CHD (31).

FGF-21, a member of the fibroblast growth factor family, is an important endocrine regulator that mainly acts through induction of weight loss and management of insulin signaling, as well as management of glucose and lipid metabolism (32). It also has important anti-inflammatory roles in various tissues/cells, such as obese adipose tissue, cardiac tissue, and macrophages (33–37). FGF-21 gene expression was reportedly reduced in EAT from patients with multivessel CHD associated with T2DM (38), while FGF-21 expression was enhanced in EAT from patients undergoing cardiac surgery, suggesting that FGF-21 has a protective effect against cardiac surgery-related inflammation (39). Therefore, anti-inflammatory pathways related to PVAT may comprise novel targets for the prevention and treatment of various vascular diseases.

PVAT and Pro-Inflammatory Cytokines

Leptin is another rich adipokine released by adipose tissue (including PVAT), which was the first adipokine reported in the literature. Under physiological conditions, leptin mainly relies on hypothalamus and sympathetic nerve signaling to reduce appetite, increase energy consumption, and regulate glucose homeostasis, independent of insulin (40). Leptin resistance is associated with the development of hypertension and insulin resistance (40), and inflammation is an important contributor to leptin resistance (41). Classically, leptin is regarded as a pro-inflammatory cytokine, which has structural homology with other cytokines such as TNF-α and IL-6 (41, 42). Leptin plays a direct role in inflammation by inducing monocytes, leukocytes, and macrophages to produce IL-6, TNF-α, and IL-12; thus, it increases the production and migration of ROS in monocytes, as well as the production of chemokine ligands by macrophages (42, 43).

In obese sedentary mice, PVAT ring-deficient mice exhibited lower levels of circulating glucose, insulin, resistin, leptin, and TNF-α; they also demonstrated abnormal endothelial function in thoracic aorta (44). The reduction of leptin expression in PVAT may inhibit neointimal hyperplasia and vascular remodeling by inhibiting monocyte migration and VSMC proliferation (45). Gene expression profiling showed that the expression levels of IL-1β, IL-6, and leptin in patients with CHD were significantly higher in PVAT around the coronary artery than in PVAT inside the thoracic artery (46). In addition, data from patients undergoing coronary artery bypass grafting indicated that the leptin–inflammation–fibrosis–hypoxia axis plays a key role in coronary atherosclerosis pathogenesis. Compared with PVAT surrounding the anti-atherosclerotic internal mammary artery, leptin expression was significantly increased in "cardiac" PVAT surrounding the aortic root and coronary arteries (C-PVAT). This increased expression was accompanied by more obvious angiogenesis and inflammation, indicating significant increases in the numbers of platelets, endothelial cell adhesion molecule 1-positive vessels, and CD68-positive macrophages, as well as greater degrees of fibrosis and hypoxia, which may lead to an enhanced coronary atherosclerotic plaque load (47). Increased expression levels of hypoxia inducible factor-1α and Fos-like antigen 2 were observed in C-PVAT; these factors reportedly enhance leptin gene transcription (47). The findings thus far suggest that PVAT plays an important role in promoting vascular inflammation through leptin secretion.

Notably, adipocytes and macrophages in PVAT also secrete large amounts of TNF-α; these levels are higher in obese animals and people than in lean animals and lean people. TNF-α induces aortic intima-media thickening through PVAT inflammation (48). TNF-α has pro-inflammatory effects and can promote the production of other pro-inflammatory factors (e.g., IL-6, leptin, and resistin). Furthermore, MCP-1 produced by adipocytes has been identified as a potential factor for macrophage infiltration into adipose tissue (49, 50). The increased expression levels of MCP-1 and TNF-α in transplanted PVAT tissue can aggravate endothelial dysfunction and atherosclerosis in distant vessels by enhancing the inflammatory response (18). In addition, RANTES (produced by T cells, macrophages, VSMCs, ECs,and PVAT adipocytes (51–53)) is a key factor for leukocyte recruitment to sites of inflammation or infection (54). Increased RANTES expression levels in hypertensive PVAT induce T-cell chemotaxis and vascular accumulation of T cells that express RANTES receptors (55). In addition, PVAT secretes free fatty acids, resistin, visfatin, and other pro-inflammatory adipokines, which participate in the occurrence and development of vascular diseases (56–58).

ROLES OF PVAT IN VASCULAR DISEASE

Morphological, structural, and functional changes of PVAT have been investigated in major vascular lesions associated with vascular diseases such as vascular aging, atherosclerosis, hypertension, and DM-related vascular dysfunction (**Table 1**).

Vascular Aging

Aging is an independent risk factor for vascular diseases. Vascular aging is mainly characterized by blood vessel-related structural changes and dysfunction that increase with age, which culminate in age-related vascular diseases (112–114). In such diseases, the vascular wall exhibits a pro-inflammatory microenvironment associated with low-grade perivascular inflammation, which is characterized by increased secretion of pro-inflammatory cytokines and chemokines, as well as enhanced infiltration of immune cells (4). These changes promote vascular dysfunction, hinder cell metabolism, increase cell apoptosis, and contribute to the onset of vascular diseases (115). In this context, PVAT is a key factor that affects vascular and perivascular inflammation during aging (**Table 1**).

There is increasing evidence that age can affect PVAT morphology and function, increase PVAT-related inflammation, and affect the corresponding vascular activity. In rats, aging reportedly attenuated the anti-contractile effect of PVAT around the thoracic aorta, while reducing the amount of brown adipose tissue-like PVAT (59). In the mesenteric arteries of SHRSP.Z-Leprfa/IzmDmcr rats (SHRSP.ZF) with metabolic syndrome, vascular dysfunction is compensated by a PVAT-dependent mechanism, which disappears with age (60). Compared with young C57BL/6JRj mice, middle-aged mice showed more PVAT hypertrophy (61); furthermore, the mean single adipocyte area in PVAT was significantly increased, while the expression level of protein inhibitor of activated signal transducer and activators of transcription 1 (a key negative regulator of inflammation) was decreased. These effects may contribute to age-related vascular diseases (61).

With increasing age, resident stromal cells in PVAT (PVASCs) exhibit decreasing differentiation ability, which contributes to neointimal hyperplasia and vascular remodeling after PVAT transplantation into carotid artery. This may be caused by the loss of PGC1α in aged PVASCs, which can be improved by overexpression of PGC1α (62). Senescence-accelerated prone mice (SAMP8), a mouse model of aging, shows vascular dysfunction associated with hypertension and cognitive decline (116). Compared with control senescence-accelerated resistant mice (SAMR1), aged SAMP8 mice reportedly demonstrated the lack of an anti-vasoconstrictive effect of PVAT; they also exhibited increased tunica media thickness, decreased APN expression, and enhanced expression levels of vascular markers of inflammation (e.g., endothelin-1, inducible nitric oxide synthase, and cyclooxygenase 2) (63).

Arterial stiffness is an inevitable result of aging. Local PVAT homeostasis, especially inflammation in PVAT, is associated with the development of age-related arterial stiffness. Loss of functional PVAT can enhance arterial stiffness in aging mice; furthermore, aged C57BL/6J mice that were fed a high-fat diet demonstrated significant induction of PVAT hypertrophy and enhancement of arterial stiffness. This change is related to the low level of mitoNEET expression in PVAT, which increases the expression of pro-inflammatory genes (64). In addition, older arteries are more susceptible to obesity-induced aging, compared with younger arteries (65). Aging aggravates obesity-induced

TABLE 1 | Central roles of PVAT inflammation in vascular diseases.

	Vessels	PVAT	Reference
Vascular aging	• Increased tunica media thickness • Increased oxidative stress and inflammation (ET-1, iNOS, COX2) • VSMCs proliferation • Increased perivascular fibrosis • Increased arterial stiffness	• PVAT was hypertrophic and the average area of single adipocyte was significantly increased • The differentiation ability of PVASCs decreased • Increased proinflammatory mediators (TNF-α, IL-6, eotaxin, MIP-1α, MCP-1 and RANTES) • Reduction of ant-inflammatory mediators (APN) • The function of anti- vasoconstriction is weakened • The infiltration of macrophages and natural killer cells	(59–68)
Atherosclerosis	• The infiltration of macrophages, T cells and dendritic cells increased • Plaque volume increased, internal lipid increased, high macrophage density and fibrin deposition • Increased proinflammatory mediators • Increased perivascular inflammation	• The size of adipocytes was smaller and the differentiation phenotype was less • Increased proinflammatory mediators (TNF-α, IL-6, IL-1β, MCP-1, resistin, and osteoprotegerin) • Reduction of ant-inflammatory mediators (APN) • The infiltration of macrophages, T cells and dendritic cells increased • B-1 cells and their secretion of anti-atherosclerotic IgM decrease	(10, 18, 69–77) (67, 78–86)
Hypertension	• Increased proinflammatory mediators (MCP-1, RANTES and MIP-1α) • Endothelial dysfunction • Increased vascular tone • Systolic and diastolic blood pressure increased • vascular hypertrophy and fibrosis • Increased perivascular inflammation	• The function of anti- vasoconstriction is weakened • Decreased production of vasodilator factor • Increased angiotensin II secretion • Increased proinflammatory mediators (IFN-γ, RANTES, C3, C5a, MCP-1, TNF-α, IL-6, MIP-1α) • Reduction of ant-inflammatory mediators (APN) • The infiltration of macrophages, T cells and dendritic cells increased • The number of eosinophils decreased	(17, 55, 68, 72, 87–97)
Diabetes mellitus related vascular dysfunction	• Increased insulin resistance • Impaired vasodilation and vascular remodeling mediated by insulin • The adhesion ability of endothelial cells to lymphocytes increased	• PVAT phenotype changed to pro-inflammatory, pro oxidative and pro vasoconstrictive state • Increased infiltration of M1 macrophages and dendritic cells • Overproduction of proinflammatory cytokines(IFN-γ, TNF-α, and IL-6) • Reduction of anti-inflammatory cytokines (IL-10 and APN)	(98–102)
Abdominal aortic aneurysm	• Recruitment of inflammatory cells (macrophages, lymphocytes, and mast cells) in vascular wall • Increased expression of perivascular inflammatory factors • Enhanced leukocyte- fibroblast interaction in adventitia • Migration and proliferation of adventitial fibroblasts increased	• Increased PVAT deposition • Co-localization of PVAT inflammation and abdominal aortic aneurysm • Increased gene expression of proinflammatory factors (IL-8, PTPRC, LCK, and CCL5) • Decreased expression of anti-inflammatory PPARγ	(103–111)

The table lists changes in PVAT and vascular inflammation during the onset of vascular aging, atherosclerosis, hypertension, diabetes mellitus-related vascular dysfunction and abdominal aortic aneurysm (note that the anti-atherosclerotic effects of healthy PVAT are not listed). ET-1, endothelin-1; iNOS, inducible nitric oxide synthase; COX2, cyclooxygenase 2; VSMCs, vascular smooth muscle cells; PVASCs, resident stromal cells in PVAT; APN, adiponectin; PTPRC, protein tyrosine phosphatase receptor type C; LCK, lymphocyte-specific protein tyrosine kinase; PPARγ, peroxisome proliferator-activated receptor gamma.

PVAT inflammation, promotes secretion of pro-inflammatory factors by PVAT (including cytokines such as TNFα and IL-6, as well as chemokines such as eotaxin and MIP-1α), and reduces APN secretion, thus increasing vascular oxidative stress and inflammation in a paracrine manner, and stimulating VSMC proliferation (65, 66).

The effect of PVAT on vascular senescence depends on its secretion, as well as the presence of inflammatory cells. Studies in humans have shown that age and body mass index are associated with the density of CD68-positive macrophages in PVAT (67). In spontaneously hypertensive rats, aging is associated with increased numbers of infiltrating leukocytes, macrophages, and natural killer cells in PVAT, accompanied by gradual elevation of blood pressure. Dual pharmacological inhibition of Nox1 and NOX4 increases blood pressure and leads to the accumulation of immune cells in PVAT. These effects are related to increased expression levels of MCP-1 and RANTES in PVAT, which lead to enhancement of perivascular fibrosis and acceleration of vascular aging (68). Overall, the existing evidence shows that PVAT mediates changes associated with vascular aging by enhancing infiltration of multiple inflammatory cells and release of various pro-inflammatory factors.

Atherosclerosis

Vascular diseases (e.g., myocardial infarction and cerebral infarction) are often caused by atherosclerosis, a vascular disease with robust inflammation, which is characterized by the accumulation of lipids, diseased cells, and necrotic debris.

Proinflammatory leukocytes and cytokines play important roles in various stages of atherosclerotic plaque formation (117). There is increasing evidence that perivascular inflammation contributes to multiple stages of atherosclerosis; notably, PVAT plays an important role in triggering adventitial inflammation in atherosclerosis. Furthermore, PVAT promotes atherosclerosis in basic vessels through "from the outside to the inside" signal transduction. However, PVAT exhibits a nonuniform role in the development of atherosclerosis. PVAT may have dual effects (i.e., both pro- and anti-atherosclerosis), which may influence balance in the local environment.

Healthy PVAT plays a protective role in regulating metabolism, inflammation, and function in nearby blood vessels. Healthy PVAT may contain immune cells that impede the development of atherosclerosis (118). The absence of PVAT can lead to enhanced macrophage infiltration and increased pro-inflammatory cytokine production in the aortic perivascular area, thus exacerbating vascular inflammation and atherosclerotic lesions in aortic wall tissue (119). In addition, PVAT is the main source of aorta-associated B lymphocytes. Many of these B cells belong to the anti-atherosclerotic B-1 subgroup; IgM secreted by this subgroup of B cells can reduce the effects of pro-inflammatory cytokines produced by M1 macrophages. Notably, the ratio of B-1/B-2 cells is reportedly 10-fold higher in PVAT than in spleen or bone marrow, indicating an important anti-inflammatory effect of PVAT (120).

The number of B-1 cells secreting anti-atherosclerotic IgM is reportedly reduced in PVAT from apolipoprotein E-/- (ApoE-/-) mice, which significantly aggravates atherosclerosis in the aorta and coronary artery (69). A systemic endocrine mechanism also mediates the anti-atherosclerotic effect of PVAT. The transplantation of PVAT from thoracic aorta of wild mice was able to reduce the atherosclerotic plaque size in suprarenal aorta of ApoE-/- mice that were fed a high-cholesterol diet. This anti-atherosclerotic effect was mediated by a transforming growth factor-β1-induced anti-inflammatory response, which may involve alternatively activated macrophages (121). In addition, APN derived from PVAT can inhibit carotid collar-induced carotid atherosclerosis by promoting macrophage autophagy (122).

In the context of chronic hyperthermia, PVAT dysfunction exacerbates atherosclerosis and increases the risk of plaque rupture. In dysfunctional PVAT, the secretion of anti-inflammatory factors (e.g., APN) is decreased, while the secretion of pro-inflammatory cytokines is increased; thus, the distribution of pro-inflammatory and anti-inflammatory immune cells is unbalanced. These changes lead to the enhancement of local inflammation, aggravating the development of atherosclerosis (**Table 1**).

Pathological conditions (such as altered expression of angiotensin II [Ang II] or pro-atherosclerotic factors) increase the dedifferentiation of PVAT adipocytes (70, 71). In larger vessels associated with atherosclerosis, adipocytes in PVAT are usually smaller and exhibit a less differentiated phenotype (10, 72, 73). Inflammation in PVAT and adventitia occurs prior to endothelial dysfunction and atherosclerotic plaque formation (74). In human aorta, PVAT accumulates in sites where atherosclerosis can easily form, while inflammatory cells concentrate in PVAT at the edge of adventitia and secrete chemokines that can attract monocytes and T cells to the adventitial interface, suggesting that PVAT promotes vascular inflammation (75). Compared with non-diseased aorta, inflammatory cells exhibited significantly increased infiltration in PVAT around atherosclerotic aorta (123). During the onset of atherosclerosis in ApoE-/- mice, macrophages, T cells, and dendritic cells were recruited into the adventitia and PVAT (75, 76), in a manner influenced by age (75). In addition, compared with subcutaneous adipose tissue transplantation in mice, carotid artery transplantation of PVAT reportedly causes large lipid rich atherosclerotic lesions in thoracic aorta, as well as high macrophage density and fibrin deposition. The inhibition of leukocyte ligand P-selectin glycoprotein ligand 1 may provide a therapeutic method to reduce the effects of PVAT inflammation on atherosclerosis (77). PVAT expansion and inflammation in obesity can remotely induce endothelial dysfunction and aggravate atherosclerosis. Transplantation of PVAT from the abdominal aorta of mice that were fed a high-fat diet promoted inflammation (increased expression of TNF-α and MCP-1; decreased expression of APN), endothelial dysfunction, and atherosclerosis in thoracic aorta, suggesting that enhanced inflammation is the potential mechanism by which PVAT exhibits a distal vascular effect (18).

Data from human samples showed that the densities of B lymphocytes and macrophages in PVAT around atherosclerotic plaque increased with plaque size; the corresponding inflammation increased with increasing coronary artery occlusion (67). The number of macrophages in PVAT was also associated with the number of immune cells in plaque (78–80). In addition, the density of macrophages was higher in PVAT near unstable plaque than in PVAT near the stable plaque. The inflammation was stronger in PVAT near stenotic sites and acute lesions than in adipose tissue distant from lesions, in the absence of atherosclerosis (67).

Pro-atherosclerotic mediators derived from dysfunctional PVAT may comprise another mechanism underlying human vascular atherosclerosis (81). Analysis of dysfunctional PVAT has revealed upregulated expression of pro-inflammatory genes, as well as downregulated expression of anti-inflammatory adiponectin (82–84). EAT from patients undergoing coronary artery bypass grafting showed significantly higher levels of chemokines (MCP-1) and pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α), compared with levels in subcutaneous adipose tissue from the same patients (84). Furthermore, the expression of anti-inflammatory APN was significantly lower in EAT samples from patients with severe coronary atherosclerosis than in EAT samples from patients without coronary atherosclerosis (83), suggesting an inflammation imbalance in PVAT from patients with atherosclerosis. The colocalization of macrophages and resistin (an adipokine that can enhance endothelial cell permeability *in vitro*) in human PVAT indicates that PVAT may participate in the pathogenesis of atherosclerosis through various mechanisms (82, 85). Osteoprotegerin, a member of the TNF-related family, is associated with atherosclerotic progression and increased instability; its expression is strongly upregulated in human perivascular adipocytes (124).

Importantly, vascular wall-associated inflammation also affects the dynamic balance of PVAT. In the presence of coronary artery inflammation and atherosclerosis, the release of pro-inflammatory mediators from the vascular wall to the surrounding PVAT leads to altered adipocyte differentiation and intracellular lipid formation, which greatly influences cardiovascular diagnosis and contributes to distinctive imaging findings (86). Noninvasive detection of PVAT can provide structural information and distinguish unstable atherosclerotic lesions. In postmortem studies of human patients, atherosclerotic plaque size and complex lipid core composition were positively associated with PVAT volume and macrophage infiltration (79). Because PVAT inflammation is related to disordered adipocyte differentiation and reduced lipid content in adipocytes, Antonopoulos et al. examined the perivascular CT fat attenuation index (FAI), a water to fat ratio index with good sensitivity and specificity in the differential diagnosis of PVAT inflammation (86). Importantly, they found that the perivascular FAI was greater in unstable plaque than in stable plaque; it was greatest near inflamed coronary arteries. PVAT imaging can provide spatial location information regarding the human coronary arteritis microenvironment, which enables early identification of high-risk plaques and may facilitate further treatment.

Hypertension

Inflammation is an important factor involved in hypertension, which involves high blood pressure and can cause both end organ damage and dysfunction (125, 126). The main site of initial inflammation in hypertension is reportedly within PVAT and the PVAT/adventitia boundary (55, 127). Inflammation leads to the loss of the anti-contractile effect of PVAT, potentially because of adipose tissue dysfunction (128). The production of vasodilators derived from PVAT adipocytes decreases during inflammation, while pro-inflammatory adipokines increasingly infiltrate into the adjacent vascular system (5); these changes enhance vascular inflammation and vascular resistance (68). PVAT inflammation leads to vascular dysfunction in the context of hypertension. Various inflammatory cells participate in this process, which is mediated by a series of cytokines and chemokines; for example, interferon-γ is produced by CD8+ cells infiltrating PVAT (55, 87), RANTES mediates the infiltration of T cells into perivascular space (55), and complement C5a mediates decreased APN production (17). These inflammation-related changes exacerbate the pro-inflammatory crosstalk and dysfunction between PVAT and hypertensive vessels (**Table 1**).

The infiltration and activation of macrophages dispersed in PVAT are important contributing factors in hypertension-related inflammation. The expression of complement C3 is reportedly increased in PVAT from DOCA-salt hypertensive mice (88), resulting in increased expression of pro-inflammatory M1 macrophage phenotype markers and decreased expression of anti-inflammatory M2 macrophage phenotype markers. Bone marrow-specific C3 deficiency significantly improved DOCA-salt–induced hypertensive vascular hypertrophy and fibrosis (89). Further studies in DOCA-salt hypertensive mice showed that the recruitment of macrophages in PVAT promotes complement activation, induces perivascular inflammation, and increases the production of TNF-α, thereby causing APN downregulation. This is a potential risk factor for hypertension-related vascular inflammation and injury (17), which may be particularly important when considering treatment methods for hypertension-related vascular injury. Moreover, in mice with spontaneous hypertension induced by perilipin-1 deletion, PVAT exhibited reduced APN expression, whereas it exhibited enhanced expression of MCP-1, TNF-α, and IL-6; additionally, the anti-contractile effect of PVAT was lost. These effects were associated with an increased pro-inflammatory response, as well as higher systolic and diastolic blood pressures in aorta (90).

Increased activation of the renin–angiotensin–aldosterone system (RAS) is an important factor in hypertension pathogenesis. With the exception of renin, almost all components of the RAS system are expressed in PVAT, implying key roles in the regulation of hypertension-related perivascular inflammation (72, 129). Lee et al. found that the release of PVAT-derived relaxing factor (PVATRF) from PVAT in spontaneously hypertensive rats was significantly reduced, while the release of Ang II was enhanced (91). In Ang II-induced hypertensive mice, the numbers of leukocytes, T cells, macrophages, and dendritic cells in PVAT were significantly increased (55, 92). In those mice, Ang II significantly increased the expression levels of MCP-1, RANTES, and macrophage inflammatory protein 1α (MIP-1α, also known as CCL3) in aorta and PVAT. Furthermore, the activation of angiotensin type 1 receptor (AT1 receptor) in PVAT promotes vascular inflammation and endothelial dysfunction (93, 94). Aldosterone may directly promote a pro-inflammatory phenotype in PVAT. Macrophage infiltration and increased C5a expression were detected in adipose tissue from patients with aldosterone-secreting adenoma; these findings were associated with decreased APN expression (17).

Hypertension was more common in obese individuals than in lean individuals. The progress of hypertension is related to the immune response of adipose tissue (130). The anti-vasoconstrictive properties of healthy PVAT are eliminated in obesity, resulting in increased arterial tension, which is a key mechanism of obesity-related hypertension and vascular dysfunction. Macrophage infiltration in PVAT plays a key role in obesity-related hypertension (95). In mice that were fed a high-fat diet, macrophages accumulated in fat PVAT around the thoracic aorta or mesenteric artery. The absence of class A1 scavenger receptor, a key pattern recognition receptor that regulates macrophage activity, can stimulate the excessive production of vascular endothelial growth factor B in macrophages from PVAT and aorta, increase the accumulation of endothelial lipid in obese mice, and promote obesity-induced elevation of blood pressure (95). Eosinophil-deficient ΔdblGATA-1 mice reportedly lack the anti-contractile function of PVAT and show elevated blood pressure (96). Notably, Withers et al. demonstrated that obesity is accompanied by a significant decrease in the number of eosinophils present in PVAT, which may lead to a loss of its anti-contractile function (97). This effect was restored by replenishment using purified

eosinophils in vessels with intact PVAT or by the use of IL-33 to restore the number of eosinophils in PVAT (96, 97). These findings suggest that PVAT releases an eosinophil-derived soluble anti-contractile factor. This factor is dependent on B3 adrenoceptor activity and independent of other downstream signaling pathways (e.g., APN and nitric oxide) mediated by immune cells (97). Thus, targeting the number of eosinophils in PVAT may constitute a novel method for the treatment of obesity-related hypertension.

Diabetes Mellitus-Related Vascular Dysfunction

The progression of DM eventually involves the development of chronic vascular complications and associated cardiovascular diseases; this cardiovascular disease progression is the leading cause of death in diabetic patients worldwide (131). Endothelial dysfunction is the initial vascular defect in DM; it is associated with DM-related macrovascular and microvascular complications (e.g., coronary heart disease, stroke, peripheral vascular disease, diabetic retinopathy, and kidney disease), which represent the main health burden in patients with DM (132). Inflammation is a major pathophysiological process that mediates DM-related endothelial dysfunction. PVAT is presumed to serve as a mechanistic link between T2DM and vascular diseases such as atherosclerosis (133).

In the context of DM, high glucose stimulation induces PVAT transition to a pro-inflammatory (increased CRP, CCL2, and CD36), pro-oxidant, and vasoconstrictive phenotype (98, 99). PVAT inflammation can promote insulin resistance in the vascular system, resulting in impaired insulin-mediated vasodilation and vascular remodeling and subsequent onset of vascular diseases (99). PVAT obtained from obese db/db mice greatly impaired insulin-mediated vasodilation of the resistance artery in muscle, while PVAT obtained from nonobese mice promoted vasodilation of this artery (100). Furthermore, obesity and the expansion of PVAT in db/db mice cause elimination of insulin-stimulated vasodilation and recovery by blocking inflammation through inhibition of the c-Jun N-terminal kinase pathway, thus indicating a key role for inflammation in PVAT (100).

Abdominal Aortic Aneurysm (AAA)

Inflammatory cell recruitment to aortic media, macrophage activation, and pro-inflammatory molecule production are important mechanisms involved in AAA (134), which contribute to gradual thinning of the aortic media and adventitia (135). Analysis of samples from human patients has shown that AAAs are surrounded by abundant PVAT (103), and the density of PVAT is higher around the aneurysm sac in patients with aortic aneurysm than in healthy neck tissue, suggesting that the deposition of PVAT is related to AAA pathophysiology (104). Overall, PVAT plays a pro-inflammatory role in the development of AAA.

Genome-wide expression profiling has revealed colocalization of PVAT inflammation with AAA, suggesting that biological changes in PVAT may be functionally associated with AAA pathogenesis (105). Changes in PVAT phenotype and function initiate inflammatory signals, stimulating the recruitment and activation of immune cells; the soluble factors produced by immune cells cause matrix degradation, leading to the initiation and progression of AAA (105). PVAT samples from AAA patients showed increased expression of various pro-inflammatory genes, including IL-8, protein tyrosine phosphatase receptor type C, lymphocyte-specific protein tyrosine, and CCL5, accompanied by decreased expression of anti-inflammatory genes (e.g., peroxisome proliferator-activated receptor gamma) and increased degradation of extracellular matrix (106). Furthermore, adipose tissue from AAA patients can induce inflammation in healthy VSMCs from control patients, resulting in increased expression of genes involved in aneurysm formation (106). Studies in animal models have shown that PVAT-derived pro-inflammatory factors accelerate the recruitment of macrophages, lymphocytes, and mast cells in the vascular wall (93). Deletion of the AT1a receptor gene in PVAT attenuated AAA development and gelatinolytic activity, as well as the accumulation of macrophages in abdominal aorta and adipose tissue; it also contributed to macrophage polarization from a pro-inflammatory state to an anti-inflammatory state. In addition, obesity-related PVAT dysfunction reportedly promotes Ang II-induced aortic aneurysm formation by secreting platelet-derived growth factor-D (PDGF-D). Leukocyte–fibroblast interactions in adventitia enhance the recruitment and activation of local monocytes, leading to aortic aneurysm and aortic dissection (107, 108); PDGF-D stimulates the migration and proliferation of adventitial fibroblasts, as well as the expression of pro-inflammatory factors. Notably, adipocyte-specific PDGF-D transgenic mice were more likely to form aortic aneurysm after Ang II infusion, accompanied by increased adventitial inflammation and fibrosis (109). Although multiple studies have shown that macrophages are the key inflammatory cells mediating the formation of AAA (110), immunophenotypic analysis of advanced AAA samples infiltrating the largest expansion site demonstrated that T cells (rather than macrophages) are the main leukocyte subset in AAA; their greatest accumulation occurs in perivascular tissues such as PVAT (111). This discrepancy may be related to differences in AAA stages between studies. However, these findings clearly indicate that inflammation in PVAT and aortic wall contributes to the pathophysiology of AAA; these proposed pathways of inflammatory induction can reveal new therapeutic targets for AAA.

CONCLUSION

PVAT dysfunction is one of the main risk factors for cardiovascular diseases; PVAT is particularly important because of its close proximity to the vascular wall. Therefore, the importance of PVAT in regulating cardiovascular complications cannot be ignored. Further mechanistic research is needed; however, immune dysfunction (i.e., increased presence of pro-inflammatory mediators, rather than anti-inflammatory mediators) and subsequent chronic inflammation play key roles. Crosstalk between PVAT and vascular system occurs in both

directions, and it has important roles in vascular homeostasis and disease. In particular, inflammation leads to PVAT dysfunction through inflammatory cells and various pro-inflammatory factors, thereby exacerbating altered vasodilation, while enhancing vasoconstriction and vascular remodeling; these changes contribute to vascular aging, atherosclerosis, hypertension, and other vascular diseases. In animal and human studies, PVAT dysfunction has been shown to cause various inflammatory vascular diseases, and vascular inflammation is associated with changes in PVAT phenotype; these findings can help to identify vulnerable vascular lesions. Although the mechanism is not entirely clear, the existing evidence shows that PVAT inflammation is a strictly regulated process that occurs in the early stage of vascular disease, which can serve as a valuable target for future treatment. Therefore, further research is needed to explore whether PVAT can be targeted in novel treatments for vascular diseases.

In addition, PVAT-related secretory factors (e.g., adipokines, hormones, and other factors) have important effects on many aspects of the vascular system. PVAT dysfunction promotes the dedifferentiation of perivascular adipocytes, such that they no longer serve as lipid storage cells; in contrast, they become metabolically active synthetic tissues, produce pro-inflammatory cytokines and chemokines, and play key roles in cardiovascular disease-related inflammation (5). Overall, extensive analysis of various adipokines is needed to clearly distinguish the physiological and therapeutic effects of these adipokines in the context of vascular dysfunction.

AUTHOR CONTRIBUTIONS

YL and YZ designed the manuscript and wrote part of it, while YC wrote most of it. ZQ revised and corrected the manuscript, and YW and XL completed the design and drawing of figure and table. All authors contributed to the article and approved the submitted version.

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REVIEW
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COVID-19 and Obesity: Role of Ectopic Visceral and Epicardial Adipose Tissues in Myocardial Injury

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In March 2020, the WHO declared coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a global pandemic. Obesity was soon identified as a risk factor for poor prognosis, with an increased risk of intensive care admissions and mechanical ventilation, but also of adverse cardiovascular events. Obesity is associated with adipose tissue, chronic low-grade inflammation, and immune dysregulation with hypertrophy and hyperplasia of adipocytes and overexpression of pro-inflammatory cytokines. However, to implement appropriate therapeutic strategies, exact mechanisms must be clarified. The role of white visceral adipose tissue, increased in individuals with obesity, seems important, as a viral reservoir for SARS-CoV-2 *via* angiotensin-converting enzyme 2 (ACE2) receptors. After infection of host cells, the activation of pro-inflammatory cytokines creates a setting conducive to the "cytokine storm" and macrophage activation syndrome associated with progression to acute respiratory distress syndrome. In obesity, systemic viral spread, entry, and prolonged viral shedding in already inflamed adipose tissue may spur immune responses and subsequent amplification of a cytokine cascade, causing worse outcomes. More precisely, visceral adipose tissue, more than subcutaneous fat, could predict intensive care admission; and lower density of epicardial adipose tissue (EAT) could be associated with worse outcome. EAT, an ectopic adipose tissue that surrounds the myocardium, could fuel COVID-19-induced cardiac injury and myocarditis, and extensive pneumopathy, by strong expression of inflammatory mediators that could diffuse paracrinally through the vascular wall. The purpose of this review is to ascertain what mechanisms may be involved in unfavorable prognosis among COVID-19 patients with obesity, especially cardiovascular events, emphasizing the harmful role of excess ectopic adipose tissue, particularly EAT.

Keywords: epicardial adipose tissue, COVID-19, obesity, cardiac injury, adipose tissue, ectopic fat, inflammation, immunity

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INTRODUCTION

Since December 2019, a global pandemic of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first reported in Wuhan, China, has been raging (1). Obesity, whose prevalence is rising worldwide, is currently a major public health issue. It was soon recognized as a risk factor for worse outcomes of COVID-19 (2), including the occurrence of acute respiratory distress syndrome (ARDS), but also adverse cardiovascular events in up to 28% of hospitalized patients (3). The role of ectopic fat depots, especially increased amounts of epicardial adipose tissue (EAT), has drawn interest in the COVID-19 setting because this cardiac adiposity could fuel critical illness in patients with obesity. The purpose of this review is to ascertain what mechanisms may be involved in the unfavorable prognosis of COVID-19 patients with obesity, especially cardiovascular events, emphasizing the harmful role of excess ectopic adipose tissue, particularly EAT.

COVID-19 PATHOGENESIS—KEY POINTS

The mechanisms of SARS-CoV-2 viral transmission and pathogenesis are now better understood and may explain why some patients appear to be at greater risk of severe forms. SARS-CoV-2 infects the host cells by binding of the viral spike (S) proteins, present on the viral envelope, to cellular angiotensin-converting enzyme 2 (ACE2) receptors and then by employing cellular serine protease TMPRSS2 for S protein priming and plasma membrane fusion (4). This enables endocytosis of the virion and entry of the viral genome into the host cell cytoplasm, followed by endosomal acidification, viral replication, and shedding of virion particles (5). Type II alveolar cells, kidney cells, myocardial cells, nasal, ileum, esophagus epithelial cells, pancreatic cells, and, interestingly, adipocytes (6–8) have been identified with high ACE2 expression and could increase SARS-CoV-2 infection and replication as demonstrated in a mouse model and HeLa cells (9, 10). Infection results in cell apoptosis, which triggers the activation of pro-inflammatory cytokines and chemokines. It has been demonstrated that SARS-CoV-2-infected patients, especially those requiring admission to intensive care units (ICUs), have large amounts of pro-inflammatory cytokines than healthy patients without SARS-CoV-2 infection (11). One of the mechanisms explaining rapid disease progression could be the "cytokine storm", a dysregulated, excessive systemic cytokine release (12). Studies have shown that serum levels of IL-6, tumor necrosis factor (TNF-α), granulocyte colony-stimulating factor (G-CSF), interferon-γ-inducible protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP-1), or macrophage inflammatory protein 1-α, among others, are higher in patients with severe conditions (i.e., requiring transfer to an ICU or mechanical ventilation or who died) than in other infected patients (13, 14). Obesity is known to be associated with a state of chronic low-grade inflammation that might be a risk factor for developing a cytokine storm form during COVID-19 disease.

OBESITY: A RISK FACTOR FOR BAD COVID-19 OUTCOMES

Obesity is increasing worldwide and is today clearly recognized as a critical risk factor for various infections, post-infection complications, and mortality from severe infection (15, 16). In particular, since the 2009 influenza A H1N1 outbreak, patients with obesity have been found to be at greater risk of severe disease and have needed more mechanical ventilation (17, 18). During the COVID-19 pandemic, poor prognostic factors have emerged such as male sex, older age, diabetes mellitus, hypertension, and the presence of prior cardiovascular or respiratory disease. These factors were associated with a greater risk of developing critical or fatal conditions (2, 19). Obesity was also soon recognized as an independent risk factor associated with worse outcomes (20, 21). The United Kingdom was the first to reveal in March 2020, through a report from the Intensive Care National Audit and Research Centre (ICNARC), that two-thirds of patients who developed serious or fatal complications following infection were overweight or obese. A US study including 5,700 patients hospitalized in New York City for COVID-19 reported that the prevalence of obesity in recovered patients was twice that in the population around the hospital (41.7% *vs.* 22%) (22). A pooled meta-analysis including 19 studies showed that individuals with obesity were 113% more at risk of hospitalization ($p < 0.0001$) (23). This was confirmed by another study including 45,650 participants from nine countries worldwide and showing an odds ratio of 2.36 (95%CI: 1.37, 4.07, $P = 0.002$) for hospitalization, and 2.63 (95%CI: 1.32, 5.25, $P = 0.006$) for invasive mechanical ventilation support (24). It has also been shown that individuals with obesity are more likely to be managed in ICUs with a need for orotracheal intubation for mechanical ventilation especially if patients are young (23, 25–27). In CORONADO, a multicentric French study of COVID-19 infection in hospitalized patients with diabetes, body mass index (BMI) was the only pre-admission criterion associated with orotracheal intubation and death at D7 especially in patients younger than 75 years (28, 29). In a French cohort of 5,795 patients hospitalized for COVID-19 infection, obesity doubled mortality in all age groups (30).

ECTOPIC FAT AND ADIPOSE TISSUE DYSFUNCTION: KEY ELEMENTS IN THE COMPLICATIONS OF OBESITY

Regional distribution of adipose tissue and the development of ectopic fat are major determinants of metabolic and cardiovascular diseases (31, 32). Dysfunction of subcutaneous adipose tissue (SAT) limits its expandability and leads to ectopic fat deposition.

Adipose Tissue Dysfunction

During weight gain, adipose tissue undergoes multiple structural and cellular remodeling processes (33) leading to a dysfunctional

tissue. Firstly, during chronic positive energy balance, mature adipocytes expand, becoming hypertrophic to store more fat. If this extra energy is not used, cell numbers increase in adipose tissue, which then becomes hyperplastic (34). Hyperplastic and hypertrophic adipocytes are often hypoxic, partly explaining the development of inflammation (35). Secondly, hypoxia also induces the production of HIF-1α, which in turn leads to a potent profibrotic transcriptional program with extracellular matrix (ECM) component accumulation, leading to fibrosis and adipose tissue dysfunction (36, 37). Concurrently, immune cells infiltrate the adipose tissue, and pro-inflammatory cytokines are overexpressed (33). Under lean conditions, high M2/M1 ratio, eosinophils, and regulatory T cells, which secrete IL-4/IL-13 and IL-10, lead to an anti-inflammatory phenotype. In obesity, activation of several stress pathways such as endoplasmic reticulum stress, oxidative stress, and inflammasome (38), but also hypoxia, induces a shift in innate immunity and lymphoid cells and a modification of macrophagic signature with a rapid shift in polarization toward an M1 phenotype, associated with adipose tissue inflammation and insulin resistance (35, 39, 40). A chronic low-grade inflammation state is therefore mainly explained by immune cell imbalance in dysfunctional adipose tissue. Stressed adipocytes release free fatty acids (FFAs) and secrete chemokines that lead to inflammatory immune cell infiltration secreting pro-inflammatory cytokines (41). Intestinal microbiota dysbiosis can also trigger inflammation by activation of immune-signaling pathways (42). The dysfunction of SAT leads to the release of FFAs to peripheral organs and ectopic fat deposition such as EAT.

Epicardial Adipose Tissue and Cardiovascular Risk

In the last decade, it has been demonstrated that ectopic fat depots localized around the heart contribute to the pathogenesis of cardiovascular disease, independently of other visceral depots (43, 44). EAT is an ectopic fat depot located between the myocardium and the visceral pericardium in close contact with coronary vessels (45). With no fascia separating the tissues, local interaction and cellular crosstalk between myocytes and adipocytes can occur. EAT is an extremely active endocrine organ with a high capacity for releasing and taking up FFAs. It is thought that EAT has protective functions as a mechanical shock absorber against pulse waves, a regulator of FFA homeostasis, and, in a more recent work, a thermogenic factor (46–49). It is a major source of adipokines, chemokines, and cytokines, interacting paracrinally or vasocrinally with vascular cells or myocytes (44). Expression and secretion of pro-inflammatory cytokines (IL-6, IL-1β, MCP-1, TNF-α, etc.) have been found to be higher in EAT than in subcutaneous fat (50), partly by the upregulation of nuclear factor κB (NF-κB) and c-Jun N-terminal kinase (JNK). It was hypothesized to accentuate vascular inflammation, plaque instability *via* apoptosis (TNF-α), and neovascularization (MCP-1).

Using a pangenomic and unbiased lipidomic approach, we previously reported that EAT has a specific transcriptomic and lipidomic signature particularly enriched in inflammation, extracellular matrix remodeling, immune signaling, thrombosis, beiging, coagulation, apoptosis, and lipotoxic pathways with an enrichment in ceramides, diglycerides, and monoglycerides compared with SAT, especially in patients with coronary artery disease (CAD) (47, 51). Furthermore, we previously demonstrated that human EAT secretome induced marked fibrosis of myocardial atria through the secretion of adipo-fibrokines, such as activin A (52). Activin A was shown to be enhanced in patients with heart failure and reduced ejection fraction and was abundantly expressed in EAT of type 2 diabetes (T2D) patients with obesity (53).

EAT thickness, volume, and density can be assessed by various imaging techniques such as echocardiography (54), computed tomography (CT), and magnetic resonance imaging (55). Higher EAT volume and lower density were associated with coronary calcification and serum levels of plaque inflammatory markers (56). EAT has been shown to be associated with CAD and the occurrence of major adverse cardiovascular events in many studies (57–60). It is correlated with the extent and severity of CAD, chest pain, unstable angina, and coronary flow reserve (61, 62) and could be a marker of the atherosclerotic burden even in asymptomatic patients (63, 64). EAT may also play a role in the development of atrial fibrillation (AF) (65) by infiltration of adipocytes in the atrial myocardium, mechanical effect on left atrial pressure stretch and wall stress, fibrosis, and inflammation, which can lead to structural and electrical remodeling and cardiac automatic system activation (44).

Obesity thus leads to an increase in ectopic fat deposition, particularly at the epicardial level, which may partly explain the increase in adverse cardiovascular events in this condition. Moreover, the pro-inflammatory phenotype of adipose tissue makes this organ a target for further immune amplification by external pathogens, such as SARS-CoV-2. In the current context of COVID-19 infection, we will see how dysfunction of the adipose tissue leads to a higher risk of severe-form COVID-19.

DYSFUNCTIONAL ADIPOSE TISSUE IN OBESITY: A KEY TO UNDERSTANDING BAD OUTCOMES DURING THE COVID-19 PANDEMIC

Immune and Metabolic Derangement as a Possible Link to Worse Outcomes in Obesity

It has been demonstrated that host cell entry of SARS-CoV-2 depending on ACE2 receptors and overexpression of human ACE2 can increase viral infection and replication. Some studies have demonstrated that the expression of ACE2 in adipocytes is higher than that in the lungs, which can act as an important viral reservoir (7, 66). Experimental studies on mice showed an increased expression of ACE2 in adipocytes in case of a high-fat diet (67). In obesity, excess adipose tissue may thus increase SARS-CoV-2 infection and accessibility to the tissue, leading to

an increased viral systemic spread, entry, and prolonged viral shedding (68), as seen during the influenza A epidemic. After infection of host cells, the recruitment of pro-inflammatory cytokines and impaired lymphocyte T cells culminates in a cytokine storm associated with progression to ARDS and multi-organ failure (13). In severe respiratory forms, patients with COVID-19 infection showed macrophage activation syndrome. There is a depletion of lymphocytes CD4 and CD8 (69) but a higher ratio of pro-inflammatory Th17 cells and high secretion of pro-inflammatory cytokines IL-2, IL-6, and TNF-α (70, 71). In obesity, dysfunctional hypertrophic adipocytes over-produce pro-inflammatory cytokines, leading to a chronic low-grade inflammation state. This in turn causes metabolic and immune derangement, making a cytokine storm more likely (72). The dysfunction of an adaptative immune system with increased pro-inflammatory LTCD4$^+$ and impaired T-cell function could also increase this risk. In this regard, the PD-1/PDL-1 immune checkpoint could increase within the visceral adipose tissue (VAT) of individuals with obesity. PD-1 is expressed by T cells and interacts with receptor PDL-1 to inhibit cytotoxic T cell responses. A recent study showed that T cells of individuals with obesity increased PD-1 expression, leading to T-cell exhaustion and dysfunction (73). During severe COVID-19, the number of TCD4$^+$ and TCD8$^+$ is also reduced, and expression of PD-1 is increased (74). Interestingly, Alzaid et al. observed particularly low levels of cytotoxic CD8$^+$ lymphocytes and increased monocyte size and monocytopenia restricted to classical CD14^{Hi} CD16$^-$ monocytes, which were specifically associated with severe COVID-19 in patients with T2D requiring intensive care (75). Monocyte loss was accompanied by morphological alteration and a hyper-inflammatory expression profile consistent with the type 1 interferon pathway (IL-6, IL-8, CCL2, and INFB-1). This particular immunophenotype could be a clue to a better understanding of the increased risk of severe forms in individuals with obesity by the escape of SARS-CoV-2 from lysis.

More recently, a significant increase in IL-1β level in plasma was reported in COVID-19 patients (11), suggesting that the NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome might be involved in the pathogenesis of infection and lung injury. NLRP3 is a multiprotein complex present in macrophages, dendritic cells, and other non-immune cells. The activation of NLRP3 as a pivotal component of the innate immune system plays a critical role in the host defense but is also associated with metabolic and inflammatory conditions (76). During SARS-CoV-2 infection, the intense and rapid stimulation of immune system response could trigger activation of the NLRP3 inflammasome pathway and the release of its products including IL-6 and IL-1β (77), which could be involved in maintaining inflammation. Viral infection could potentiate this underlying systemic inflammatory state, which could partly explain worse outcomes in obese patients (78).

It has also been demonstrated that individuals with obesity display white adipose tissue depot in large airway walls, proportionally to BMI, which could lead to airway thickening, immune cell infiltration, and then tissue damage and fibrosis in the lungs (79, 80). Also found in the lungs, lipofibroblasts, adipose-like cells composed of lipid droplets and located in the alveolar interstitium, could transdifferentiate to myofibroblasts and lead to pulmonary fibrosis (5, 7).

There would then be a higher expression of ACE2 and TMPRSS2 in lung epithelial cells from individuals with obesity than in those without, as demonstrated *in vitro* (81).

These conditions could be another basis for the elevated occurrence of ARDS in obese individuals with obesity.

These different elements partly explain the role of adipose visceral tissue in critical COVID-19 infection, as a viral reservoir and by increasing immune responses with consequences for cytokine cascade amplification and severe forms of the disease. VAT and EAT could be markers of severity, and recent studies also show that it could be implicated in myocardial injury.

Visceral Adipose Tissue and Epicardial Adipose Tissue as Markers of Myocardial Injury

Cardiac complications have been reported in 28% of patients hospitalized for COVID-19 infection (3, 82, 83). Myocardial injury and myocarditis with elevated troponin occurs in 7%–17% of hospitalized patients and are associated with an increased risk of adverse outcomes (84, 85). Acute myocarditis represents a significant diagnostic challenge because of its varied clinical presentations and risk of worse outcomes such as heart failure. Changes in electrocardiograms, elevated cardiac biomarkers, and impaired cardiac function should be considered as alerts pointing to acute myocarditis (86). Remarkably, no culprit injury was found in 40% of patients with COVID-19 presenting ST-elevation myocardial infarction (87), which could be promoted by hypercoagulability, endothelial dysfunction, microvascular damage, hypoxia-induced injury, myocarditis, or systemic inflammatory cytokine storm syndrome. In several studies, cardiac troponin I level was found to be associated with more severe disease and mortality, making myocardial damage a prognostic factor (88, 89). Furthermore, dysrhythmias linked to hypoxia, inflammatory stress, and therapeutics affect up to 17% of hospitalized patients (90, 91). Finally, some studies report that heart failure may be present in 23% of patients hospitalized for COVID-19, half of whom had no history of hypertension or cardiovascular disease.

The mechanisms of these cardiac events are not fully clarified, and ectopic fat and EAT could be important triggers of their development. More than just BMI, several reports have shown that VAT volume measured by CT is associated with critical illness in patients with COVID-19 entailing hospitalization (92), intensive care need, or death (93–96). According to Favre et al., a visceral fat area ≥128.5 cm^2 was the best predictive value for severe COVID-19 (93). Further, EAT, known to be strongly correlated with VAT, has been associated with the occurrence of cardiac events in COVID-19 infection.

CT imaging of the EAT allows adipose tissue inflammation to be characterized by quantifying CT threshold attenuation.

The group of Iacobellis showed that density of EAT, reflecting inflammatory changes, significantly increased with increasing COVID-19 severity compared to discharged patients (97). Furthermore, EAT mean attenuation was negatively correlated to high-sensitivity troponin T levels and peripheral oxygen saturation (97). Another international multicenter study on 109 patients showed that volume and attenuation of EAT measured by CT was associated with extent of pneumonia and were independent predictors of clinical deterioration or death (98). This study used a fully automated three-dimensional measurement of EAT and demonstrated that EAT volume can predict clinical deterioration or death independently of clinical factors such as age, diabetes, hypertension, or smoking history (99). This suggests the importance of automated measurement of EAT for COVID-19 risk stratification. An increased EAT volume was associated with lung dysfunction even in healthy individuals (100), and the close proximity of EAT to the pulmonary circulation could enable direct diffusion of inflammatory mediators. According to Wei et al., EAT volume appeared to be an independent predictor of myocardial injury in patients with COVID-19 (OR = 3.06) with a maximal cutoff value of 137.1 cm^2 (89) after adjustment for age, weight, history of cardiovascular disease, and dyslipidemia. This work performed in a large cohort of 400 patients from six Chinese hospitals clearly indicates that EAT volume enlargement may predict the development of myocardial injury. However, the cutoff needs to be evaluated in ethnically diverse cohorts. Furthermore, EAT was significantly higher in severe cases of COVID-19 groups, i.e., with signs of respiratory distress (101). In a recent study, Iacobellis et al. showed that on 427 infected patients, use of dexamethasone reduced EAT attenuation (102). EAT could therefore also serve as a therapeutic target for anti-inflammatory treatment. All these studies indicate that EAT volume and inflammation itself are associated with COVID-19 severity and adverse cardiac events.

The mechanisms of these cardiac events are not fully elucidated, and EAT could be a clue to understanding them. First, epicardial fat cells seemed to express higher levels of ACE2 than subcutaneous fat cells, which could make them a viral reservoir in COVID-19 infection. A study on EAT and SAT biopsies from 43 patients who underwent open-heart surgery identified higher levels of ACE2 ($p < 0.05$) but lower ADAM-17 ($p < 0.001$), with its cleavage enzyme in EAT compared with subcutaneous fat. Obesity and T2D exacerbated this difference in patients with cardiovascular disease (103). In an animal study, ACE2 was upregulated in murine EAT in association with high-fat diet. Loss of ACE2 in knock-out diet-induced-obesity (ACE2KO-DIO) mice increased macrophage polarization to a pro-inflammatory phenotype and EAT inflammation compared with wild-type and control diet mice. The same study showed that in human EAT from obese patients with heart failure, ACE2 was increased and was also associated with pro-inflammatory macrophage phenotype compared with lean patients (104, 105). Voluminous and hypervascularized EAT in individuals with obesity could facilitate viral spread, immune response, and greater pro-inflammatory cytokine secretion. Volume of EAT was positively correlated with inflammatory biomarkers during COVID-19 infection in a study of 100 patients (106), with a significant positive mild association with neutrophil-to-lymphocyte ratio ($r = 0.33$, $p = 0.001$) and platelet-to-

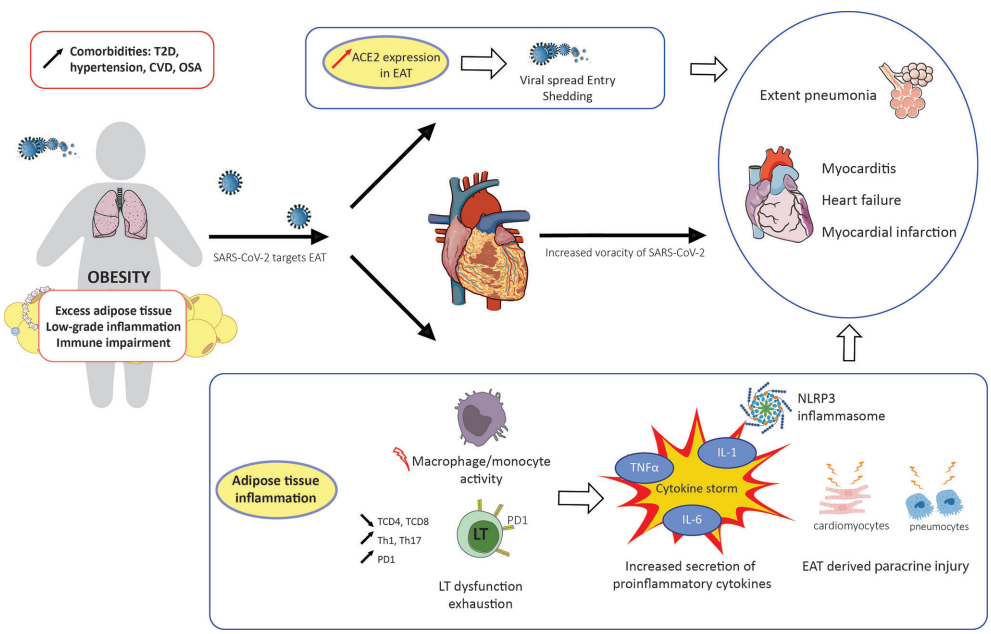

FIGURE 1 | Impact of obesity and inflammation of epicardial adipose tissue on COVID-19 outcome. CVD, cardiovascular disease; EAT, epicardial adipose tissue; OSA, obstructive sleep apnea; T2D, type 2 diabetes.

lymphocyte ratio (r = 0.25, p = 0.01) but a negative correlation with lymphocyte-to-C-reactive protein (CRP) ratio (r = −0.25, p = 0.02). Pro-inflammatory cytokines such as TNF-α and IL-6 are expressed at higher levels in EAT of individuals with obesity linked to a reduction of inotropic effect and cardiac function resulting in hypoxia and systemic myocardial inflammatory response (43). By taking advantage of more ACE2-binding sites, which ultimately lead to an augmented inflammatory signaling cascade, EAT inflammation could contribute to myocardial complications, such as myocarditis or cardiomyocyte dysfunction (107), and then heart failure. Furthermore, it has recently been shown that EAT adipocytes can release exosomes that can enter cardiac cells *via* endocytosis (105). This suggests numerous mechanisms by which EAT could impair cardiac function, particularly *via* the transfer of microRNAs from EAT to the myocardium and could help mediate SARS-CoV-2 entry into the heart, causing direct cardiac effects.

COVID-19 thus induces an immune-mediated inflammatory response, and EAT may transduce this inflammation to the heart. It can be implicated in COVID-19 myocarditis by its contiguity with the myocardium and its pro-inflammatory secretome reaching the myo-pericardium directly by the vasa vasorum and paracrinally (108–110).

EAT thus contributes to bad outcomes during COVID-19 infection. We and others have shown that EAT significantly responds to drugs targeting the fat (44). EAT not only is a marker of inflammation, but it can be a target to anti-inflammatory treatment. Further studies on the impact of COVID treatment on EAT volume and inflammation are needed.

All these elements are summarized in **Figure 1**.

CONCLUSION

Obesity is a major risk factor for COVID-19. Identifying patients with obesity who are at high risk of ICU need is crucial. Multiple studies have demonstrated that ectopic fat accumulation, especially EAT, is a major driver of COVID-19 severity in such patients. This unique potentially inflamed EAT depot may play a direct role in COVID-19 cardiac injury, acting as a fuel through its specific anatomical contact with the myocardium and its inflammatory status. Large studies with systematic evaluation of EAT volume and CT scan attenuation together with evaluation of pulmonary involvement are needed. Deep learning algorithms leading to new fully automated three-dimensional methods for the measurement of EAT will help improve clinical risk stratification.

AUTHOR CONTRIBUTIONS

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REVIEW
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Gut Microbiota in Adipose Tissue Dysfunction Induced Cardiovascular Disease: Role as a Metabolic Organ

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The gut microbiome has emerged as a key regulator of host metabolism. Accumulating evidence has indicated that the gut microbiota is involved in the development of various human diseases. This association relies on the structure and metabolites of the gut microbiota. The gut microbiota metabolizes the diet ingested by the host into a series of metabolites, including short chain fatty acids, secondary bile acids, trimethylamine N-oxide, and branched-chain amino acids, which affects the physiological processes of the host by activating numerous signaling pathways. In this review, we first summarize the various mechanisms through which the gut microbiota influences adipose tissue dysfunction and metabolic processes that subsequently cause cardiovascular diseases, highlighting the complex interactions between gut microbes, their metabolites, and the metabolic activity of the host. Furthermore, we investigated the current status of clinical therapies for adipose tissue dysfunction directed at the gut microbiota. Finally, we discuss the challenges that remain to be addressed before this field of research can be translated to everyday clinical practice.

Keywords: gut microbiota, adipose tissue dysfunction, molecular endocrinology, cardiovascular disease(s), gut dysbiosis

Abbreviations: ANGPTL2, Angiopoietin-Like Protein 2; ATD, Adipose Tissue Dysfunction; BAs, Bile Acids; BCAAs, Branched-Chain Amino Acids; BMI, Body Mass Index; BS, Bariatric Surgery; CA, Cholic Acid; CDCA, Chenodeoxycholic Acid; CVDs, Cardiovascular Diseases; CYP7A1, Cholesterol 7α-Hydroxylase; DCA, Deoxycholic Acid; FGF15, Fibroblast Growth Factor 15; FMO, Flavin Monooxygenase; FMT, Fecal Microbiota Transplantation; FXR, Farnesol Nucleus Receptor; GC, Gas Chromatography; GUDCA, Glycoursodeoxycholic Acid; HDAC, Histone Deacetylase; HFD, High-Fat Diet; IL, Interleukin; LBP, Lipopolysaccharide Binding Protein; LC, Liquid Chromatography; LCA, Lithocholic Acid; LPS, Lipopolysaccharide; MAMPs, Microbial-Associated Molecular Patterns; MD2, Myeloid Differentiation Protein 2; MS, Mass Spectrometry; mTORC1, mammalian Target Of Rapamycin Complex 1; MYD88, Myeloid Differentiation Primary Response 88; NGS, Next Generation Sequencing; NO, Nitric Oxide; PKCε, Protein Kinase Cε; PXR, Pregnane X Receptors; RBP-4, Retinol Binding Protein 4; RYGB, Roux-en-Y Gastric Bypass; S1PR2, Sphingosine-1 Phosphate Receptor 2; SAT, Subcutaneous Adipose Tissue; SCFAs, Short-Chain Fatty Acids; sFAs, saturated Fatty Acids; SG, Sleeve Gastrectomy; SGLT1, Sodium Glucose Cotransporter-1; T2D, Type 2 Diabetes; TGF-β, Transforming Growth Factor β; TGR5, Takeda G Protein-Coupled Receptor-5; TLR, Toll-Like Receptor; TMA, Trimethylamine; TMAO, Trimethylamine-N-Oxide; TNF-α, Tumor Necrosis Factor α; VAT, Visceral Adipose Tissue; VFA, Volatile Fatty Acids; 16S rRNA, 16S ribosomal RNA.

1 INTRODUCTION

Obesity is a major global health concern. There is a general consensus that obesity increases the risk of cardiovascular diseases (CVDs). By far the most widely accepted measure of obesity is the body mass index (BMI), a simple calculation based on an individual's weight in kilograms divided by the square of their height in meters (kg/m^2) (1). A high BMI has been associated with shorter life expectancy (2), which is mainly due to increased risk of type 2 diabetes (T2D), hypertension, dyslipidemia, and CVD (3).The Global Burden of Disease study group estimated that elevated BMI values were responsible for 4 million deaths in 2015, with two thirds of this number attributed to CVDs (4). However, it has been pointed out that the BMI alone cannot fully identify patients at high-risk of CVDs. Wildman et al. (5) found that a substantial proportion, approximately 50% of overweight individuals and 30% of obese individuals, are free from any obvious signs of metabolic or cardiovascular complications. Furthermore, CVDs are also common in people with normal BMI, which suggests BMI is a highly heterogeneous indicator. That is because BMI is an overall indicator related only to height and weight, which does not take body composition into account. At an individual level, BMI can neither distinguish between fat and lean tissue nor determine fat distribution, function, and associated risk factors (1).

Adipose tissue dysfunction (ATD) or "adiposopathy" is a relatively recent concept and is thought to be more closely associated with CVDs than the BMI (6). In the past, the adipose tissue was thought to be relatively inert and its only function was as a storage depot for excess energy in the form of triglycerides and to build up or break down excess lipid into free fatty acids and glycerol based on the metabolic needs of the body. However, growing research suggests that adipose organs are considered to be quite active tissues with metabolic functions and are involved in crosstalk between multiple organ systems (7).

In recent years, a significant number of studies have been conducted investigating the role of gut microbiota in various endocrine and immune diseases, such as obesity, diabetes, nonalcoholic fatty liver disease, allergies, and CVDs (8, 9). There has been much literature summarizing the relationship between gut microbiota and CVDs, involving a wide range of gut microbiota metabolites and their endocrine functions *in vivo (*10, 11). To date, the gut microbiota is considered a novel endocrine organ that effects many metabolic activities in the host. In this review, we provide a brief review of the gut microbiota and its metabolites, and describe the endocrine roles and molecular mechanisms involving the gut microbiota and metabolites in various aspects of ATD-induced CVDs. Finally, we also briefly review the treatment of various aspects of ATD-induced CVDs targeting the gut microbiota and thus, provide guidance for future research in the emerging field of the gut microbiome associated with the development of metabolic diseases in humans.

2 ADIPOSE TISSUE DYSFUNCTION

This concept of ATD further details the phenotype of obese patients. ATD consists of 2 aspects: abnormalities in fat distribution and abnormalities in the characteristics of adipose tissue, which are both highly related to CVDs (**Figure 1**).

Fat distribution abnormalities refer to the excessive accumulation of visceral adipose tissue (VAT), or fat storage in the intraperitoneal and retroperitoneal spaces, and deposition of ectopic fat, fat stores in body locations where fat is not physiologically stored such as the liver, pancreas, heart, and skeletal muscle. Normally, adipose tissue accumulates in subcutaneous adipose tissue (SAT, 80-90%) (12) and the main depots of SAT are the abdominal, subscapular (on the upper back), and gluteal and femoral areas (13, 14). The SAT depot is located under the skin and does not communicate with internal organs. It is considered as a normal physiological buffer for excess energy intake with little threat to cardiovascular health (15). Conversely, through excessive energy intake, sex hormones levels (16), use of glucocorticoids (17), genetic make-up (18), and epigenetic mechanism (19), fat tends to store in the intraperitoneal and retroperitoneal areas, and in locations where it is not physiologically stored such as the liver, pancreas, heart, and skeletal muscle, which has been defined as VAT. Ross et al. (20) found that individuals matched for abdominal SAT with low or high VAT levels had different levels of glucose tolerance, whereas those matched for VAT had similar glucose tolerance testing with high and low SAT. This study indicated that SAT may not be a risk factor for metabolic diseases, whereas VAT and ectopic fat accumulation was causally related with insulin levels. In addition, several studies have reported positive associations of excess VAT accumulation with cardiovascular risk factors, CVDs and all-cause mortality (21–23). Furthermore, adipose tissue accumulation at special ectopic sites such as the pericardium or epicardium also results in increased CVD risk (24).

Another aspect of ATD involves abnormalities in the characteristics of adipose tissue. Adipocytes typically constitute the majority of the cellular content of adipose tissue. Adipocytes are surrounded by fibrous connective tissue, collagen, nerves, and blood vessels. Despite its location, the metabolic activity of adipose tissue is also a determinant of ATD-induced CVDs. Features of dysfunctional adipose tissue include impaired adipose tissue expandability and adipogenesis (20), as well as hypertrophy and altered lipid metabolism by fat cells. Most importantly, macrophage infiltration can be observed in ATD and initiates a vicious cycle of inflammatory response (25), leading to polarization of macrophages toward a pro-inflammatory phenotype (M1-polarized), which can activate inflammatory pathways and impair insulin signaling (26). In addition, adipokines, the bioactive compounds which are synthesized in the adipose tissue and released into circulation, can be dysregulated in ATD. This dysregulation is a prominent hallmark of ATD (27). Expression of pro-inflammatory adipokines such as leptin, tumor necrosis factor α (TNF-α),

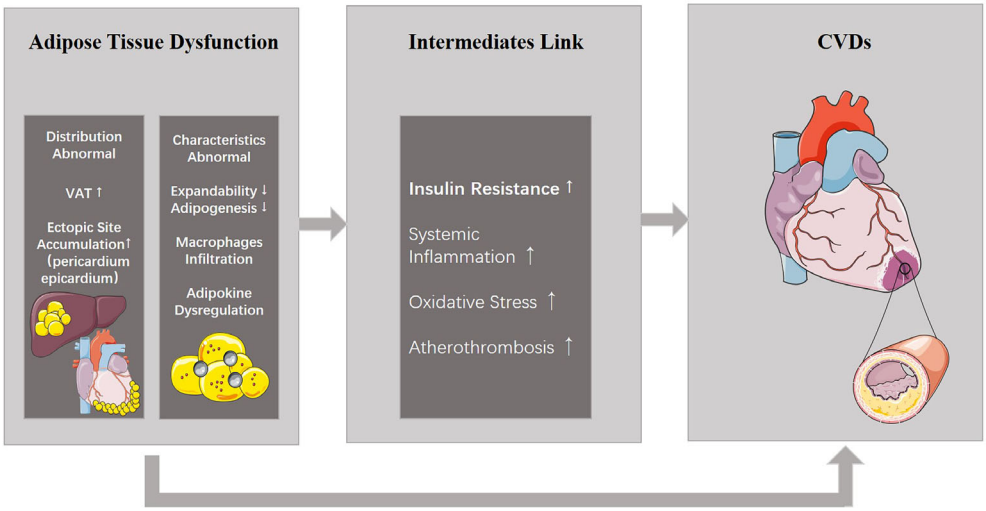

FIGURE 1 | ATD and its relationship with CVDs. ATD consists of two aspects: abnormal distribution and properties. ATD is able to induce CVDs directly, or results in other metabolic diseases including insulin resistance which eventually leads to CVDs. ATD, adipose tissue dysfunction; CVDs, cardiovascular diseases.

interleukin-6(IL-6), interleukin-8(IL-18), retinol binding protein 4, lipocalin 2, and angiopoietin-like protein 2 far exceeds that of anti-inflammatory factors such as adiponectin, IL-10, and nitric oxide (NO). The imbalance between pro- and anti-inflammatory cytokines in favor of pro-inflammatory ones leads to insulin resistance, systematic inflammation, oxidative stress, atherothrombosis and eventually, CVDs (28). However, systemic inflammation probably involves a complex network of signals interconnecting several organs. The causal relationship between systemic inflammation and adipose tissue inflammation is currently controversial. In summary, ATD increases cardiovascular risk through adipose tissue ectopic accumulation and dysregulation of adipokines in adipose tissue.

3 GUT MICROBIOTA AND METABOLITES

3.1 Gut Microbiota—an Endocrine Organ

The term gut microbiota describes the commensal microbial species in the gastrointestinal tract (29). Trillions of microbial cells including bacteria, fungi, archaea, and viruses are harbored in the human intestine, which act as an essential and complicated part of our healthy physiological ecosystem. With a bacterial load of more than 10^{14} (30), the genome carried by the gut microbiota outnumbers human genome by 100 times (31). More than 90% of the taxa of the gut microbiota in adults is constituted by two major bacterial phyla, Bacteroidetes or Firmicutes, with a lower abundance of Actinobacteria, Cyanobacteria, Fusobacteria, Proteobacteria, and Verrumicrobia (32). Collectively, these bacteria make up the most complex and diverse ecosystem in human gut. Although the composition of gut bacteria is remarkably similar at the phylum level and a core of bacterial genera is present in the majority of adult hosts, there is huge variability at a subspecies level. The human gut microbiome is also highly dynamic and can be dramatically altered by age, antibiotic use, host genetics, chronic dietary patterns, and other environmental exposures (31, 33, 34).

Gut microbiome plays a predominant role in training and maturation of the host immune system (35), vitamin synthesis, resistance to colonization by or overgrowth of pathogenic microorganisms, deconjugation of bile acids (BAs) (36), and energy harvest through fermentation of indigestible carbohydrates (37). With this huge microbiome, diversity at the subspecies level, it is not surprising that the gut microbiota has a significant influence on the biological activity of the host. Indeed, the gut microbiota plays an important role in the regulation of metabolic activities of the human body, which is mainly achieved through its metabolites (38).

The Merriam-Webster dictionary defines an endocrine organ as follows: "producing secretions that are distributed in the body by way of the bloodstream." Besides endocrine organs in the conventional sense like the hypothalamus, pituitary gland, thyroid gland, and adipose tissue, the gut microbiome also fits this classic definition. But unlike host endocrine organs, which produce only a few key hormones, the gut microbial endocrine organ has the unique potential to produce hundreds if not thousands of humoral agents defined as metabolites of gut microbiota. These metabolites are sensed by highly selective host receptor systems that elicit diverse biological responses (39), and finally, alter the metabolic functions of distal organs.

3.2 Metabolites of Gut Microbiota

The gut microbiota produces a wide range of metabolites, such as vitamins and short-chain fatty acids (SCFAs), BAs, trimethylamine (TMA), branched-chain amino acids (BCAAs), ammonia, and phenols (8). These metabolites are absorbed by intestinal epithelial cell and finally enter the circulation. In addition, structure components of microbiome like

lipopolysaccharide (LPS) could also enter the circulatory system of the host and become bioactive compounds (**Figure 2**). These microbiota-derived metabolites and LPS are agents of microbe-host communication, which is essential to maintain vital functions of the healthy host (39).

3.2.1 Short-Chain Fatty Acids

SCFAs, also known as volatile fatty acids (VFA), are the end product of the gut microbiome's fermentation of indigestible carbohydrates (such as fructose-oligosaccharides, inulin, oligosaccharides, non-starch polysaccharides, and oat bran) (40). SCFAs are a group of organic fatty acids with carbon chains between 1 and 6. They mainly include acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, iso-valeric acid, iso-caproic acid, and hexanoic acid. The content of acetic acid, propionic acid and butyric acid is the highest in the intestinal tract: acetic acid (40–100 mmol/kg), propionic acid (15–40 mmol/kg), butyric acid (10–30 mmol/kg), the latter is the main SCFA in the intestinal tract (41). For healthy individuals, several factors affect the production of SCFAs, including the source and chemical properties of the substrate, the type and number of intestinal microorganisms, and the time of transportation in the intestine (42).

SCFAs have a variety of biochemical and physiological effects. First, SCFAs can be used for the biosynthesis of lipids, cholesterol, and proteins. Second, SCFAs act as signaling molecules to distant tissues and organs of the host. The effects of SCFAs are in part mediated by G-protein coupled receptors (GPR41, GPR43, and GPR109A) and histone deacetylase (HDAC), which are related to oxidative stress, the immune response, insulin resistance, and inflammatory responses (43, 44).

3.2.2 Bile Acids

BAs are a general term for a large group of cholic acids that exist in the form of sodium salts or potassium salts. Primary BAs are synthesized from cholesterol in hepatocytes through multiple reactions such as hydroxylation, side chain oxidation, isomerization and hydrogenation, and finally forms chenodeoxycholic acid (CDCA) and cholic acid (CA). Further, primary BAs combine with glycine or taurine in the liver to form conjugated BAs, which enter the gallbladder. After food ingestion, the gallbladder releases these BAs into the duodenum, where they function in the small intestine and colon. After the BAs assist in the digestion of food, most are reabsorbed back into the liver in a process known as the enterohepatic circulation of BAs (45). A number of recent studies have found that unabsorbed BAs in the intestine can be used as substrates for microbial metabolism and further transformed into secondary BAs through hydrolase or dehydrogenase enzymes. These intestinal bacteria are mainly composed of *Lactobacillus, Streptococcus, Enterobacter, Enterococcus, Clostridium, Lactobacillus, Veronococcus* (46). They separate BAs from glycine or taurine to form mainly deoxycholic acid (DCA) and lithocholic acid (LCA), which in turn, may be further recirculated to the liver and, like primary BAs, combine with glycine or taurine and are excreted in small amounts in the feces (45).

BAs have many important physiological functions. Their function was first recognized as an emulsion to promote the absorption of fatty acids and fat-soluble vitamins in the human body. With their further study, BAs have attracted much attention as a signaling molecule for diverse endocrine and paracrine functions (47). BAs are able to bind to the G-protein coupled receptor sphingosine-1 phosphate receptor 2 (S1PR2)

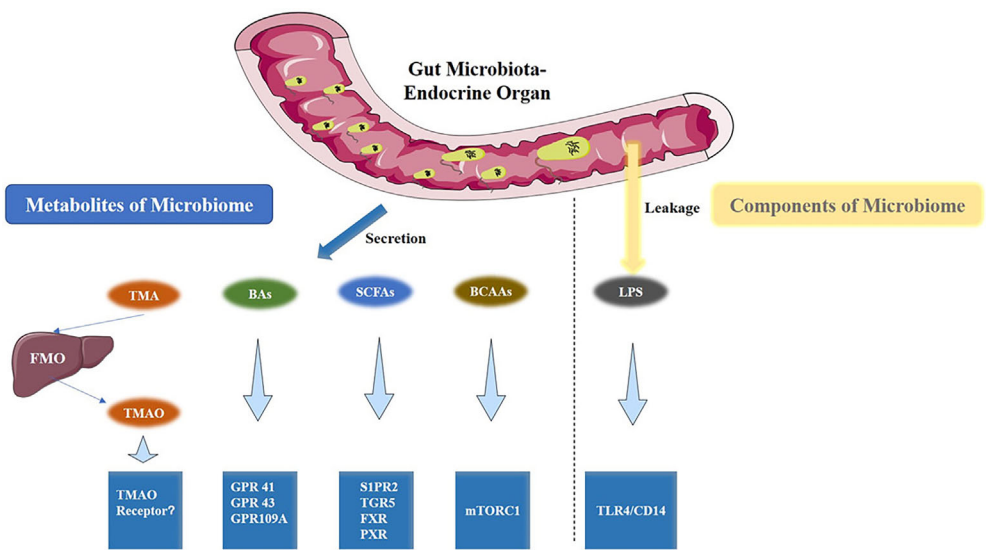

FIGURE 2 | Gut microbiota metabolites and the associated metabolic signaling pathway. TMAO, BAs, SCFAs, BCAAs are the metabolites that are produced by the gut microbiota. Conversely, LPS is a component of the cell wall of gut bacteria. All are capable of activating specific signaling pathways. TMAO, Trimethylamine Oxide; BAs, bile acids; SCFAs, short-chain fatty acids; BCAAs, Branched-Chain Amino Acids; LPS, Lipopolysaccharides.

(48) and the Takeda G protein-coupled receptor-5 (TGR5) (49). BA can also activate farnesol nucleus receptor (FXR) (50), which induces fibroblast growth factor 15 (FGF15) expression and inhibits the expression of cholesterol 7α-hydroxylase (CYP7A1) in the liver, the rate-limiting step in BA synthesis, leading to decreased BA levels *via* a gut-microbiota-liver feedback loop. In addition, BAs have been reported to bind to vitamin D receptors and pregnane X receptors (PXR) *in vivo*. By activating these different signaling pathways, BAs play a role in regulating liver gluconeogenesis, glycogen synthesis, insulin sensitivity, and regulating the balance of energy metabolism in the body (51, 52).

3.2.3 Trimethylamine-N-Oxide

Trimethylamine-N-Oxide (TMAO) is a monoamine metabolite found in gut microbiota that has attracted much attention because of its relationship with CVD in recent years (53). Foods containing choline, phosphatidylcholine, and L-carnitine are sources of TMA (54, 55). Choline, and its precursor phosphatidylcholine, are an abundant chemical constituents in daily diets such as animal liver, milk, and egg yolks. L-carnitine is an abundant nutrient in meat, especially red meats. Both choline and carnitine within the gut are absorbed within the small bowel *via* specific transporters, but absorption is incomplete, particularly with large meals that can saturate the uptake systems. Consequently, both dietary choline and carnitine ingestion can lead to significant elevations in TMA in the intestine. When TMA is absorbed into the circulation, it can be further delivery to the liver *via* the portal circulation, is rapidly converted into TMAO by hepatic flavin monooxygenase (FMO) (56).

Since TMAO was discovered, increasing research data, including human and animal models, has shown significant associations with a variety of diseases. Current studies have found that TMAO is closely associated with CVD (57), nephropathy (58), and diabetes (59). Although the relationship between TMAO and the above diseases has been established, the precise receptor or chemical sensor that detects TMAO remains unknown. Several possible mechanisms of TMAO in the occurrence and development of CVDs have been reported. Mechanisms mainly include (1): the inhibition of the reverse transport of cholesterol, and altering of the metabolic pathways of cholesterol and lipoprotein in the intestinal tract, blood vessel wall, liver, and other important organs (55) (2); promotion of vascular dysfunction and the inflammatory response through MAPK, NF-κB and NLRP3 inflammasome pathways (60, 61); and (3) the promotion of the release of Ca2+ in platelets, and then direct improvement of the response hypersensitivity of platelets, and acceleration of thrombosis (62).

3.2.4 Lipopolysaccharides

LPS, known as a bacterial endotoxin, is a component of the outer membrane of Gram-negative bacteria and is important for maintaining the structural integrity of the bacteria. LPS consists of three components, including O-specific polysaccharides, core polysaccharides, and lipid A, which is the toxic center of LPS. Strictly speaking, LPS is not a metabolite of the gut microbiota. However, LPS still exerts its effects as a metabolism-independent signaling molecule. Dysfunction of the host's gut such as following changes in permeability allows LPS to enter the circulation and activate several pathogenetic pathways (63).

First, LPS binds to the LPS binding protein (LBP), which transports LPS to the surface of immune cells and binds to the membrane protein receptor CD14. CD14 then transfers LPS to the Toll-like receptor 4 (TLR4) and myeloid differentiation protein 2 (MD2) protein complex. MD2 helps TLR4 recognize LPS, which activates intracellular signal transduction pathways and eventually activates the transcription factor NF-κB, generating pro-inflammatory cytokines such as TNF-α, IL-1b, and IL-6 (64).

3.2.5 Branched-Chain Amino Acids

BCAAs, including leucine, isoleucine, and valine, are essential amino acids and BCAAs or BCAA-rich diets usually have a positive effect on body weight regulation, muscle protein synthesis, and glucose homeostasis (65). The gut microbiota plays an important role in both *in vivo* synthesis and absorption of BCAAs. *Prevotella copri* and *Bacteroides vulgatus* were found significantly associated with increased BCAAs biosynthesis and decreased transport of genes at the fecal metagenomics level. *Butyrivibrio crossotus* and *Eubacterium siraeum* were found to be associated with the uptake of BCAAs (66).

It is now well established that there is a very close relationship between BCAAs and insulin resistance. BCAAs are considered to be sensitive biomarkers in plasma that can respond to the degree of insulin resistance. However, it is unclear whether BCAAs are involved in the development of insulin resistance or whether they are just indicators of the disease (65). Current studies have assumed that BCAAs are anabolic signals that alter the growth of energy-consuming tissues, mediated in part through their ability to activate the mammalian target of rapamycin complex 1 (mTORC1) and protein kinase Cε (PKCε) (67), which may explain the bioactivity of BCAA in insulin resistance (67).

3.3 Techniques Used in Microbial Analysis

Although the identification methods of gut bacteria and their metabolites are not the focus of this review, a brief description of the common methods is necessary. Initially, bacterial culture was the only method to identify and analyze the gut microbiota (68), but with the advent of new techniques, it is current use is in the isolation and cultivation of a single bacterium, which is still important in establishing a link between bacteria and diseases (69). In addition, culture of single bacterium allows researchers to edit genes of a specific bacteria and to analyze its characteristics (70).

3.3.1 Next Generation Sequencing

Emerging next generation sequencing (NGS) methods have replaced bacterial culture as the most efficient way to obtain information on all species of the human microbiome and even their respective genomes (71). To date, 2 common NGS strategies have been used in gut microbiota research: 16S

ribosomal RNA (16S rRNA) gene sequencing and whole genomic shotgun sequencing (72).

Sequencing of 16S rRNA genes is currently the most widely used sequence-based technique for bacterial taxonomic and phylogenetic studies. It involves sequencing of specified microbial amplicons (mainly 16S rRNA). 16S rRNA genes are present in all gut microbiomes, encoding RNAs composed of small bacterial ribosomal subunits of the 30S. The16S variable regions are specific to bacteria and 16S rRNA gene sequencing has great potential for determining genera or species. Although 16S rRNA gene sequencing has important applications in bacterial identification and classification, its low resolution limits its application. In addition, 16S rRNA generally provides information about the composition of the microbiome, but lacks functional annotation (73).

Whole-genome shotgun sequencing means using high-throughput genome sequencing combined with advanced computational bioinformatics to identify taxonomic and potentially functional microbiomes. Theoretically it can bypass microbiome culture and identify the function of microbial communities, but it still requires more experimental confirmation (74).

3.3.2 Mass Spectrometry Chromatography

In addition to the identification of the composition of the gut microbiota, the identification of the metabolites of the gut microbiota is equally very important. Mass spectrometry (MS), including tandem MS, is an important method for identifying metabolites of gut microbiota. Its high sensitivity allows us to analyze trace amounts of bacterial metabolites (75). MS is often coupled with chromatographic technology including gas chromatography (GC) and liquid chromatography (LC). This method combines the advantages of separation capabilities of chromatography and sensitivity of MS, reducing matrix effects and ion suppression that allows for a more accurate quantification and compound identification (76). This method is the most advanced technically, and most of the bacterial metabolites such as BA, SCFA, TMAO, LPS can be analyzed using this method (75, 77).

Furthermore, after identifying the metabolites of bacteria, functional omics techniques are used to link gut microbiota composition, metabolites, and diseases together. These techniques include transcriptomics, proteomics, and metabolomics (78).

4 GUT MICROBIOTA IN ATD INDUCED CVDs

4.1 "Gut Dysbiosis"

As mentioned above, although the gut microbiota is relatively stable in healthy individuals, it constantly changes throughout the life in response to endogenous and exogenous factors. From the moment intestinal bacteria are established in the infant, its composition and abundance are constantly modified under the influence of various factors such as delivery method, food, age, and exposure to antibiotics (79). Although the gut microbiota is constantly changing, its function in the maintenance of host homeostasis remains stable within a certain range in healthy humans.

'Gut dysbiosis' refers to a morbid change in the composition of the gut microbiota and may be caused by several factors such as diet, increased stress, inflammation, and antibiotic use (80). Although there is not a clear cause-and-effect relationship between this change and disease, dysbiosis can be observed in a wide range of diseases. A classic example of gut dysbiosis is the change in *Firmicutes/Bacteroidetes* ratio. As the two most abundant phylum of gut microbiota, changes in *Firmicutes/Bacteroidetes* ratio have been associated with a variety of diseases, such as obesity, gallstone disease, hypertension (81). For example, Emoto et al. reported a characteristic change in microbial composition of patients with coronary artery disease in which there was a significant increase in *Lactobacillales* (*Firmicutes*) and a decrease in *Bacteroidetes* (82). Currently, emerging evidence has shown that gut dysbiosis is able to disturb homeostatic functions of many metabolic activities through alteration of its community structure and metabolites (83). Thus, gut dysbiosis is considered a basic process of gut microbiota influencing disease development such as adipose tissue dysfunction (84), insulin resistance (66) and CVD.

However, gut dysbiosis involves alterations in a wide range of bacteria and their metabolites, so its relationship with adipose tissue dysfunction induced CVD is intertwined (**Figure 3**). Below we will explain the role of gut dysbiosis in the development of each aspect of the disease.

4.2 Gut Dysbiosis and Adipose Inflammation

The relationship between high-energy diet and obesity is unquestionable, and moreover, there is evidence that the intake of specific substances, such as saturated fatty acids (sFAs), induces adipose tissue dysfunction. With increasing understanding of the gut microbiota, scientists found that ATD is not purely a consequence of specific diet, but rather, may require the disturbance of intestine-microbiota interaction (85).

As mentioned, adipose inflammation is the most important feature in ATD. It is upstream of systemic inflammation and systemic insulin intolerance which leads to CVDs. Tran et al. (86) demonstrated that gut dysbiosis induced by a western diet is responsible for adipose inflammation in mice: an increase in classic proinflammatory M1 macrophages, and a decrease in anti-inflammatory M2 macrophages in adipose tissue were observed. Ablation of the gut microbiome could reduce such inflammatory responses in adipose tissue. Moreover, gene depletion of Toll-like receptor (TLR)-signaling adaptor protein myeloid differentiation primary response 88 (MyD88) largely phenotyped microbiota ablation, supporting the notion that western diet-induced adipose inflammation did not result from lipid accumulation per se, but rather was promoted by microbiota or their products activating innate immune signaling pathways. Although the study did not examine the metabolites of gut microbiota, it confirmed that gut microbiota

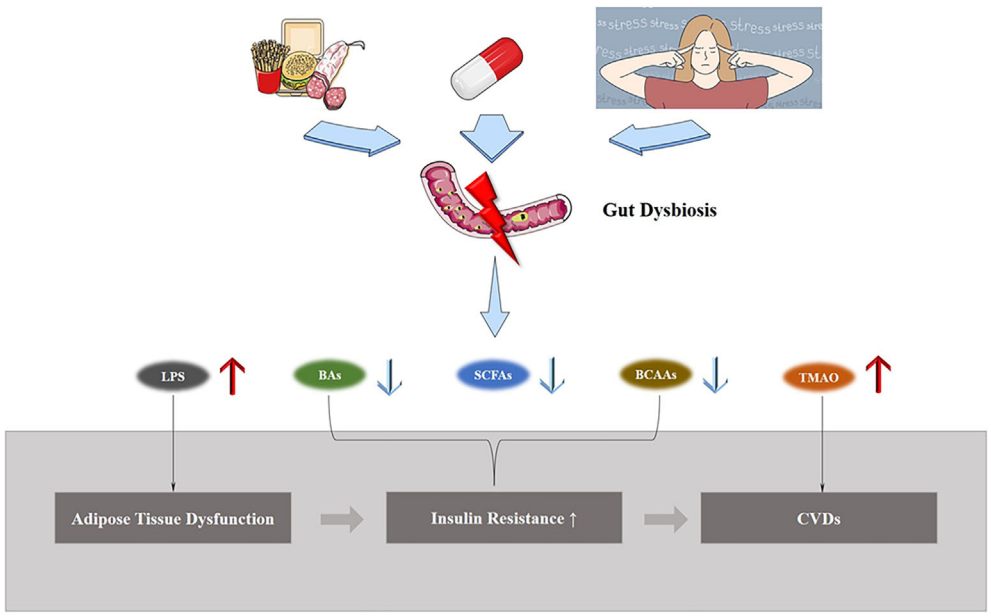

FIGURE 3 | Gut dysbiosis and ATD induced CVDs. Exposure to external environment changes, such as a high-fat diet, treatment with antibiotics, and stress, lead to gut dysbiosis, causing changes in the structure of gut microbiota and metabolite levels, and increases the morbidity of ATD-induced CVDs.

and its metabolites induce adipose tissue inflammation through an inflammatory response related to TLRs. Previously, many researchers had long demonstrated that LPS was able to activate TLR (87) and LPS had been shown to be highly correlated with the development of obesity (88). The above findings could indicate a significant correlation between LPS produced by the gut microbiota and adipose tissue inflammation.

Moreover, recent studies are more supportive of the concept that dysbiosis of gut microbiota is an early event in inflammation and the development of obesity (84). When eating a high-fat diet (HFD), the first to be exposed to these nutrients is gastrointestinal system (89). A HFD induces gut dysbiosis and alters the secretory patterns of gut peptides. These changes can provoke an increase in the intestine mucosal inflammatory response, a disruption of the epithelial barrier, and a consequent enhanced transit of LPS into the systemic circulation.

LPS, as well as sFA consequent to a HFD intake may act in synergy to promote a harmful proinflammatory response as they are recognized as endogenous ligands for specialized TLRs, normally activated following the recognition of microbial-associated molecular patterns (MAMPS) by macrophages or intestinal epithelial cells (90), leading to a systemic low-grade inflammation. In recent years, researchers have found that TLR can also be expressed in adipose tissue, and have also found TLR-bearing macrophages resident in adipose tissue (91). Upon activation of TLRs, macrophages in adipose tissue are induced to the M1 pro-inflammatory phenotype and subsequently generate various inflammatory mediators (92). Adipose tissue may also secrete pro-inflammatory adipokines such as TNF-α, IL-6, IL-8, and MCP-1 in a TLR-dependent manner in response to LPS (93, 94). On initiation, this inflammatory environment may further recruit new inflammatory cells (neutrophils, macrophages), thus magnifying the inflammatory response in adipose tissue. Overall, systemic low-grade inflammation can promote adipose inflammation, leading to ATD, which in turn can further exacerbate systemic inflammation and insulin resistance, ultimately leading to CVDs.

4.3 Gut Dysbiosis and Insulin Resistance

Insulin resistance refers to a condition where the pancreas produces insulin, while muscle, liver, and other cells fail to respond to insulin, which leads to glucose intolerance and eventually diabetes, which is the most important link between ATD and CVDs. In parallel to ATD, gut dysbiosis can also directly mediate insulin resistance. In humans with obesity, a 7-day treatment with vancomycin, an antibiotic against Gram-positive bacteria, can modulate the composition of the gut microbiota and decrease peripheral insulin sensitivity (95). Adjustment in the Firmicutes/Bacteroidetes ratio has been proposed in insulin-resistant patients by many researches (96). In addition, intestinal bacterial species producing SCFAs such as *Roseburia*, *Eubacterium halii*, and *Faecalibacterium prausnitzii* are generally decreased, while opportunistic pathogens such as *Lactobacilli gasseri*, *Streptococcus mutans*, and *Escherichia coli* are increased in subjects with T2D (97). Thus, the relationship between insulin resistance and changes in intestinal bacterial genera and metabolites, with a major focus on BCAAs and SCFAs, has been demonstrated in many studies.

As mentioned, BCAAs are well-known biomarkers of insulin resistance and predictors of incident diabetes and CVDs. The gut microbiota has a wide range of enzymatic functions that trigger BCAA biosynthesis and BCAA are also adsorbed and enter the

human circulation. Pedersen et al. (66) analyzed the gut microbiota and metabolites of 219 individuals without diabetes using non-targeted metabolomics and microbial genomic approaches. BCAAs were analyzed as important markers of insulin resistance. In addition, *Prevotella copri* and *Bacteroides Vulgatus* were found significantly associated with increased BCAAs biosynthesis in insulin resistance patients. Another study (98) also found that *P. copri* was the strongest driver species for the positive association between microbial BCAAs biosynthesis in the gut and insulin resistance, and *P. copri* was significantly increased in individuals receiving a HFD. Interestingly, Kovatcheva-Datchar Petia et al. (99) reported a contrasting conclusion between glucose intolerance and *P. copri* using a different dietary regimen, high in fiber and low in fat. Nevertheless, there is reason to believe that diet-induced gut dysbiosis influences the development of insulin resistance through changes in BCAA composition.

SCFAs, such as butyrate, acetate and propionate, display beneficial effects on peripheral tissues, such as adipose tissue, liver tissue, and skeletal muscle tissue, leading to an improvement in insulin sensitivity (100). SCFAs, as ligands, bind to FFAR2 and FFAR3 receptors distributed on enteroendocrine L-cells and play a role in regulating blood glucose. FFAR2 promote the secretion of insulin, GLP-1, GIP, and PYY secretion, promote the release of growth hormone, and reduce insulin signaling in fat. As for FFAR3, it promotes the secretion of GLP-1 and PYY, promote gluconeogenesis in the intestine, and decrease insulin secretion (101). In addition, increased permeability is associated with translocation of bacteria and their cell wall components which triggers an inflammatory cascade that has been associated with insulin resistance. SCFAs appear to play an important role in maintaining the integrity of the intestinal epithelial barrier through regulation of tight junction proteins, thus protecting the host from insulin resistance (102). When gut dysbiosis occurs, the SCFA-producing microbiome tends to decline, resulting in weaker regulation of blood glucose and reduction of insulin resistance by SCFAs, which in turn increases the risk of insulin resistance and even diabetes in the host (97).

BAs are also involved in glucose metabolism. Tauroursodeoxycholic acid is reported to have the potential to improve liver and muscle insulin sensitivity in obese hosts (103). Whist glycoursodeoxycholic acid (GUDCA) has the potential for treating hyperglycemia (104). Sun et al. (105) revealed that ablation of the gut microbiome alleviates HFD-induced glucose intolerance, hepatic steatosis, and inflammation by modulating the key enzyme CYP7A1 in the alternative BA synthesis pathway in hamsters, which indicates the dysbiosis of gut microbiota may contribute to insulin resistance by altering BA metabolism.

4.4 Gut Dysbiosis and Cardiovascular Diseases

Significant data has accumulated on the relationship between gut dysbiosis and CVDs. Extensive studies have demonstrated the role of gut dysbiosis in different CVDs, such as atherosclerosis (106), hypertension (107), and thrombosis (62). Numerous clinical trials have also demonstrated that many alterations in intestinal bacteria are significantly associated with the development of CVDs. *Collinsella, Proteobacteria, Actinobacteria, Enterobacteriaceae, Lactobacillales, Escherichia coli*, and *Klebsiella spp*, as well as the *Firmicutes/Bateriodetes* ratio, which has been reported to increase in CVD, as well as Butyrate-producing bacteria such as *Roseburia intestinalis*, while *Faecalibacterium cf. prausnitzii* has been reported to decrease in CVDs (82, 108–111). Notably, in ATD and insulin resistance, there is a clear causal relationship between gut dysbiosis and disease risk or severity, that is, such dysbiosis is the cause of disease onset. However, in heart failure, a CVD disease, there is evidence that the development of heart failure is responsible for the dysbiosis of gut bacteria (112). The mechanism might be the edema of the intestinal wall caused by systemic congestion secondary to heart failure systemic congestion. In turn, the highly permeable intestinal mucosa impaired the intestinal barrier function, leading structural components of microbiota (majorly LPS) to enter the circulation and to stimulate host immune responses and vascular inflammation, which causes gut dysbiosis to aggravate heart failure (113). Clinical trials have proven that enhanced abundance of pathogenic microbial were isolated from fecal samples of HF patients, especially in those with right ventricle heart failure and impaired intestinal barrier function (114).

Another common CVD, atherosclerosis, is also associated with gut dysbiosis. Koren et al. first reported the changes of gut microbiota were associated with atherosclerosis using 16S rRNA genes to survey bacterial taxa (115). At that time, whether these changes represented only microbial taxa associated with coronary heart disease or whether these alterations were risk factors that could promote the development of coronary heart disease has not been fully determined. With more contemporary studies to be reported, Jie et al. (116) observed an increased abundance of *Enterobacteriaceae* and oral cavity-associated bacteria and relatively depleted butyrate-producing bacteria in patients with atherosclerotic CVD *versus* those in healthy control subjects, suggesting that gut dysbiosis may be associated with the development of atherosclerosis. To date, the bulk of evidence suggests that gut dysbiosis is a significant risk factor for atherosclerosis.

TMAO, which links gut dysbiosis to atherogenesis, is one of the most important discoveries of recent years in the study of gut microbiota metabolites. In 2011, Wang et al. (53) identified a strong relationship between TMAO and the risk of CVD risks in a cohort study of approximately 2000 patients with atherosclerosis using a non-targeted metabolomics approach. This study suggested that level of plasma TMAO was strongly associated with future development of major adverse cardiovascular events (death, myocardial infarction, and stroke) in atherosclerosis patients. The mechanisms involved in TMAO-induced atherosclerosis have been described in previous sections. Notably, the value of TMAO is currently focused on prognostic value of circulating TMAO levels. Wang et al. (117). confirmed that TMAO predicted major adverse cardiac events in a 3-year follow-up cohort, even in the presence of elevated levels

of non-microbial metabolites. Furthermore, the prognostic value of TMAO was also observed in patients with heart failure (118), diabetes (59), peripheral artery disease (119), and chronic kidney disease (58) independently of traditional risk factors.

Other metabolites such as BAs and SCFAs can also exert an effect on CVDs, but the role they play is that of physical modulators. These affect CVDs by modulating the upstream metabolic processes, as mentioned above, ATD, and insulin resistance. This also suggests that gut dysbiosis, by producing pathogenic effects and metabolic disorders at multiple points, permeates the development of CVDs due to ATD.

5 GUT MICROBIOME-BASED THERAPEUTICS IN ATD INDUCED CVDs

As research progresses, we discover that several traditional therapies are able to influence the distribution of the gut microbiota. Many pharmaceutical effects that are difficult to explain by conventional theories can also be explained by changes in the gut microbiota. In ATD-induced CVDs, several conventional therapies have proven to be strongly associated with the modulation of gut dysbiosis.

5.1 Diet as an Important Modulator of the Gut Microbiota

What we eat, no doubt, is associated with ATD, insulin resistance, and other metabolic diseases. A breadth of knowledge exists regarding the impact of dietary fat intake on metabolic diseases (120, 121). For instance, De Souza et al. reported that consumption of HFDs correlated with insulin resistance (122). Woods et al. reported that a high fat intake is associated with increased fat storage (123). The gut microbiota serves as a filter for the largest environmental exposure—the diet. There have been several studies attempting to explain the role of the gut microbiota in the diet causing ATD, insulin resistance, and CVDs. For instance, HFDs have been associated with low-grade systemic inflammation *via* increases in circulating microbially-derived LPS. In addition, HFDs have been linked to atherosclerosis through the microbial production of TMA from L-carnitine and phosphatidylcholine. All of these findings suggest that modification of food composition may be the best way to treat metabolic disorders induced by gut dysbiosis because of its simplicity and ease of implementation.

The effects of a diet rich in saturated fat is highly likely to induce an increase in body mass, liver triglyceride content, and insulin insensitivity than a diet rich in monounsaturated fat or polyunsaturated fat by increasing the proportion of Firmicutes *versus* Bacteroidetes in the gut (124). In addition, long-term exposure to diets rich in L-carnitine and phosphatidylcholine cause excessive production of TMAO and subsequent induction of CVDs (125). In turn, by adjusting the intake of these substances, including of sFAs and choline, exercising, as well as intermittent fasting, we are better able to adjust the risk of metabolic diseases and CVDs (126, 127).

In addition, the intake of substances such as fish oil and nondigestible oligosaccharides has been shown to reduce the risk of metabolic diseases induced by gut dysbiosis. Caesar et al. (128) found that mice consuming a fish oil diet were protected from inflammation mediated by gut dysbiosis. This effect may be correlated with enrichment of the gut microbial genera *Akkermansia* and *Lactobacillus*. In addition, a high-fish-oil diet was reported to prevent adiposity and modulate white adipose tissue inflammation pathways in mice (129). Nondigestible oligosaccharides act as "fertilizers" of the gut microbiota, enhancing the growth of beneficial commensal organisms (e.g., *Bifidobacterium* and *Lactobacillus* species) (130). Cani et al. (131) found that oligofructose increased intestinal *Bifidobacteria* in HFD mice and that endotoxemia was significantly negatively correlated with *bifidobacterial* species. They also showed a significant positive correlation between *bifidobacterial* species and improved glucose tolerance and normalized inflammatory tone in high-fat oligofructose-treated mice. Neyrinck et al. (132) showed that wheat arabinoxylans in the diet counteracted high fat diet-induced gut dysbiosis with an improvement of obesity and lipid-lowering effects. In addition, the positive effects of wheat arabinoxylans including hypocholesterolemia, anti-inflammatory activity, and anti-obesity, have been associated with changes in the gut microbiota. Thus, we propose that by modifying food composition and intake of probiotic agents, can we effectively decrease the risk of ATD induced CVDs.

5.2 Metformin Exerts Therapeutic Effects by Improving Gut Dysbiosis

Metformin is an agent commonly used in clinical practice to treat diabetes. Metformin has also been reported to correct ATD (133) and to improve prognosis of CVDs (134). Initially The antihyperglycemic effect of metformin was mainly attributed to the reduction of intrahepatic gluconeogenesis. However, as research continued, metformin was found to be inextricably linked to the biological activity of the intestine. Bailey et al. described elevated levels of metformin accumulating in the gut of diabetes patients that were 300 times that found in the plasma (135). Forslund et al. reported metformin as a key contributor to changes in the human gut microbiome composition in patients with diabetes (136). Currently, many mechanisms have been reported in metformin activity in the treatment of insulin resistance and diabetes *via* improvement of intestinal dysregulation.

First, metformin is able to shift the gut microbiota toward a SCFA-producing microbiome in T2D individuals (137). Relative abundance of more than 80 bacterial strains are altered by metformin compared to placebo, where most changes are observed in the Firmicutes and Proteobacteria phyla, leading to an increase in SCFAs. In addition, metformin was reported to enhance the abundance of *Akkermansia muciniphila* (138), a mucin-degrading bacterial strain, which has gained considerable attention because of the reduction in the abundance of this bacterium associated with obesity, insulin resistance, diabetes, and CVDs in rodents and humans (139). Second, metformin treatment increases the levels of the BA glycoursodeoxycholic acid by inhibiting *Bacteroides fragilis*, thus improving insulin

resistance by activating the FXR pathway (104). Third, metformin treatment is able to increase the abundance of Lactobacillus in the upper small intestine and restore the expression and thus increase the glucose sensitivity of the sodium glucose cotransporter-1 (SGLT1)-dependent pathway, which lowered glucose production in rodents (140).

5.3 Bariatric Surgery Alters Gut Microbiota in Metabolic Disease Patients

Bariatic surgeries (BSs) such as Roux-en-Y gastric bypass (RYGB) or sleeve gastrectomy(SG) are increasingly being used and proven to be effective treatment for diabetes and morbid obesity (141). Evidence has shown that changes in the gut microbiota are believed to play a role in metabolic improvements after BS. However, due to the small sample size of studies of gut microbiota after BS in humans, changes in the composition of the microbiota have yielded very contradictory results (142). For example, the abundance of Firmicutes was reported to be decreased in two studies (143, 144) but increased in two others (145, 146). Besides, Murphy et al. described an increase in the microbial population for Bacteroidetes after SG and a decreased population for the same phylum after RYGB (145). Meanwhile, Aron-Wisnewsky et al. (147). reported GS failed to fully rescue decreased gut microbial gene richness induced by severe obesity, despite the improvement in microbial gene richness. In any case, these contradictory results at least suggest that GS is able to exert an impact on the composition and abundance of gut microbiota.

Interestingly, an observational line study of 13 patients undergoing RYGB suggested that changes in microbial functional potential are greater than changes in microbial species abundance of the gut microbiota (148). This result reminds us that besides studying compositional changes, it is more important to pay attention to function and even metabolomics output of gut microbiota. A recent meta-analysis suggested that BCAAs were significantly decreased after BS, whilst TMAO levels were elevated in post-operative measurements. Although these changes different from those we may expect, they also suggest that a change in the level of metabolites is associated with gut microbiota alterations after BS surgery. The mechanisms induced by such changes and the effects on the organism remain to be further explored.

6 DISCUSSION

We are exposed to an enormous variety of microorganisms residing in our gut, ranging from bacteria, viruses, fungi, and archaea to phages and protozoa. The gut microbiome can modulate nutrient metabolism upon dietary intake and produces many metabolites that interact with the host in a variety of ways. In this review, we have summarized the current knowledge on the gut microbiota in relation to ATD-induced CVDs in order to broaden our understanding in this field and to move towards establishing clinical applications, which may include a better understanding of etiology, pathology, and personalized interventions.

Nevertheless, additional studies are needed to clarify a number of issues regarding the relationship between the gut microbiota and ATD-induced CVDs. First, many of the relationships between gut microbiota and ATD-induced CVDs are based on findings from animal studies. The compositional differences of gut microbial communities between humans and rodents make animal study findings not directly translatable to humans (149). Observations or studies in human populations are lacking. Secondly, current studies have simply confirmed the presence of altered endocrine properties of the gut microbiota and its metabolites in patients with ATD, but whether such change is the cause or the consequence of ATD is still undetermined. Thirdly, current novel therapies for gut dysbiosis such as fecal transplantation (FMT) not only offers great potential for the treatment of a wide array of diseases, but are also a good model to study the cause-effect relationship between gut microbiota and metabolic disorders in humans. To date, animal FMT studies have demonstrated that the gut microbiota may modulate host metabolic diseases such as obesity and insulin resistance (150). In addition to FMT, transplantation of single probiotic species such as *Lactobacillus rhamnosus GG* has been shown to improve associated CVDs at the animal level (151). It cannot be denied that these therapies are very promising, but there is a lack of evidence for their effectiveness and safety in the population. A TMA-lyase inhibitor called 3,3-dimethyl-1-butanol, which in microbial cell cultures and *in vivo* mouse models reduces TMA/TMAO production without compromising microbial cell survival is another promising therapeutic approach lacking clinical evidence (152). In addition, interactions between the gut microbiota and existing drugs for CVDs such as aspirin, clopidogrel, statins, and angiotensin inhibitors are also worth investigating. Therefore, future research is necessary to incorporate the wealth of information on gut microbiota into clinical decision pathways and personalized treatment.

AUTHOR CONTRIBUTIONS

XY and XZ declare equal contribution to this work. QT and YW conceived the task. XY, QH, SL, and ZS performed the review and collected original studies. XY and XZ wrote the first draft of the manuscript. YW and QT revised the manuscript. WY, XG, HY, and HX contributed to language editing and final revision. All authors contributed to the article and approved the submitted version.

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Association of Pericardiac Adipose Tissue With Coronary Artery Disease

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Background and Aim: Coronary artery disease (CAD) poses a worldwide health threat. Compelling evidence shows that pericardial adipose tissue (PAT), a brown-like adipose adjacent to the external surface of the pericardium, is associated with CAD. However, the specific molecular mechanisms of PAT in CAD are elusive. This study aims to characterize human PAT and explore its association with CAD.

Methods: We acquired samples of PAT from 31 elective cardiac surgery patients (17 CAD patients and 14 controls). The transcriptome characteristics were assessed in 5 CAD patients and 4 controls *via* RNA-sequencing. Cluster profile R package, String database, Cytoscape were applied to analyze the potential pathways and PPI-network key to DEGS, whereas the hubgenes were predicted *via* Metascape, Cytohubba, and MCODE. We use Cibersort, ENCORI, and DGIDB to predict immunoinfiltration, mRNA-miRNA target gene network, and search potential drugs targeting key DEGs. The predictable hubgenes and infiltrating inflammatory cells were validated in 22 patients (12 CAD samples and 10 control samples) through RT-qPCR and immunohistochemistry.

Results: A total of 147 different genes (104 up-regulated genes and 43 down-regulated genes) were identified in CAD patients. These different genes were associated with immunity and inflammatory dysfunction. Cibersort analysis showed monocytes and macrophages were the most common subsets in immune cells, whereas immunohistochemical results revealed there were more macrophages and higher proportion of M1 subtype cells in PAT of CAD patients. The PPI network and module analysis uncovered several crucial genes, defined as candidate genes, including Jun, ATF3, CXCR4, FOSB, CCl4, which were validated through RT-qPCR. The miRNA-mRNA network implicated hsa-miR-185-5p as diagnostic targets and drug-gene network showed colchicine, fenofibrate as potential therapeutic drugs, respectively.

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Conclusion: This study demonstrates that PAT is mainly associated with the occurrence of CAD following the dysfunction of immune and inflammatory processes. The identified hubgenes, predicted drugs and miRNAs are promising biomarkers and therapeutic targets for CAD.

Keywords: CAD (Coronary artery disease), PAT (pericardial adipose tissue), inflammation, macrophage cell, bioinformation

INTRODUCTION

Coronary artery disease (CAD) is a global health threat, particularly due to its high level of morbidity, which poses an enormous socioeconomic and medical burden (1). Obesity, a type of metabolic syndrome characterized by abnormal deposition of body fat with chronic inflammation of adipose tissue, is associated with multiple cardiovascular diseases, including CAD (2). According to the Framingham Heart Study, the risk factors for CAD are more associated with omental adipose tissue than subcutaneous adipose tissue (SAT) (3). These observations may be plausible owing to the differences in adipose tissue endocrine and metabolism. White adipose tissue (WAT) and brown adipose tissue (BAT) are the two major types of adipose tissues. Briefly, WAT comprises adipocytes with a large, single fat droplet and is presumed as the main depot for lipid storage, whereas BAT comprises several smaller fat droplets and numerous mitochondria and plays a role in heat production (4). In humans, BAT was thought to rapidly involute and essentially disappear within the first years after birth, only a small amount is found in the scapula, paraspinal, and around the heart and aorta in adulthood (5).

Pericardial adipose tissue (PAT), which refers to the fat surrounding the external surface of the pericardium, is supplied by the internal mammary artery (6). PAT covers the pericardium which is closely adjacent to the epicardial adipose tissue (EAT), coronary artery, and the heart. Scholars have suggested that PAT may play a vital role in cardiovascular disease (7–9). Previous reports in humans indicate that PAT may appear brown-like adipocyte in morphology, with distinct features different from WAT and BAT (10–12). Elsewhere, PAT, as a metabolically active endocrine local adipocyte depot, was found to be associated with coronary artery disease (CAD) through the production of free fatty acids and pro-and anti-inflammatory adipocytokines (13). Elevated PAT volume is known to be associated with coronary atherosclerosis, hypoadiponectinemia, inflammation and represents the highest risk factor for atherosclerosis (14). Other reports have further demonstrated the association of PAT with cardiovascular events and left ventricular remodeling (15, 16). However, whether the transcriptome of PAT changes during CAD, the molecular mechanism by which PAT mediates CAD progress, and the possibility to improve the function of PAT in CAD treatment remains elusive.

In the present study, we employed the RNA-sequencing (RNA-Seq) approach to explore the transcriptome characteristic shift in PAT from humans undergoing cardiac surgery with or without CAD. This was followed by the analysis of the functional enrichment, protein-protein interaction (PPI) network, hubgenes, and microRNA (miRNA)-mRNA regulatory network. We further predicted the potential drugs that target the key differentially expressed genes (DEGs). This study will deepen our understanding of PAT in the pathogenesis of CAD.

STUDY PATIENTS

Subject Recruitment

This study complied with the Declaration of Helsinki and was approved by the ethics committee of Huadong Hospital Affiliated to Fudan University, Shanghai, China (2020K082). All patients signed written informed consent, and underwent preoperative coronary angiography. Control patients were referred for elective valve surgery and exhibited no significant CAD (a single lesion >50%) on preoperative coronary angiograms. Besides, CAD patients were referred for coronary artery bypass (CABG) surgery because of significant stenosis and surgical indications. Eventually, 31 patients, including 17 CAD patients undergoing coronary artery bypass grafting (CABG) and 14 control patients undergoing valve replacement or valve repair were enrolled for analysis.

Sample Collection and Preparation

We took a sample of PAT (1.0 g) adjacent to the pericardial surface during surgery (**Figure 1A**). The adipose sections collected from 5 CAD and 4 control patients were kept in 10% formalin for histological analysis. The other adipose tissues were immediately snap-frozen and stored at liquid nitrogen (–196°C). Five CAD and 4 control adipose tissue samples underwent RNA sequencing, while the rest were used in RT-PCR analyses.

Histology and Immunohistochemistry

Formalin-fixed adipose tissues were embedded in paraffin and sectioned (8μm thick) and stained with hematoxylin and eosin (H&E). H&E-stained sections revealed the adipocyte size. To quantify M1 or M2 macrophages, cryosections were stained with rabbit anti-human CD11b (1:1000 dilution) (Abcam, UK) and rabbit anti-human CD68 (1:1000 dilution) (Abcam, UK) antibodies after which a goat anti-rabbit secondary antibody was conjugated to horseradish peroxidase. Immunohistochemical images were captured using a Zeiss LSM 5 Pascal microscope (M205FA, Zeiss, Oberkochen, Germany). Image-Pro Plus software (version6.0, MEDIA CYBERNETICS, USA) was employed to analyze the images.

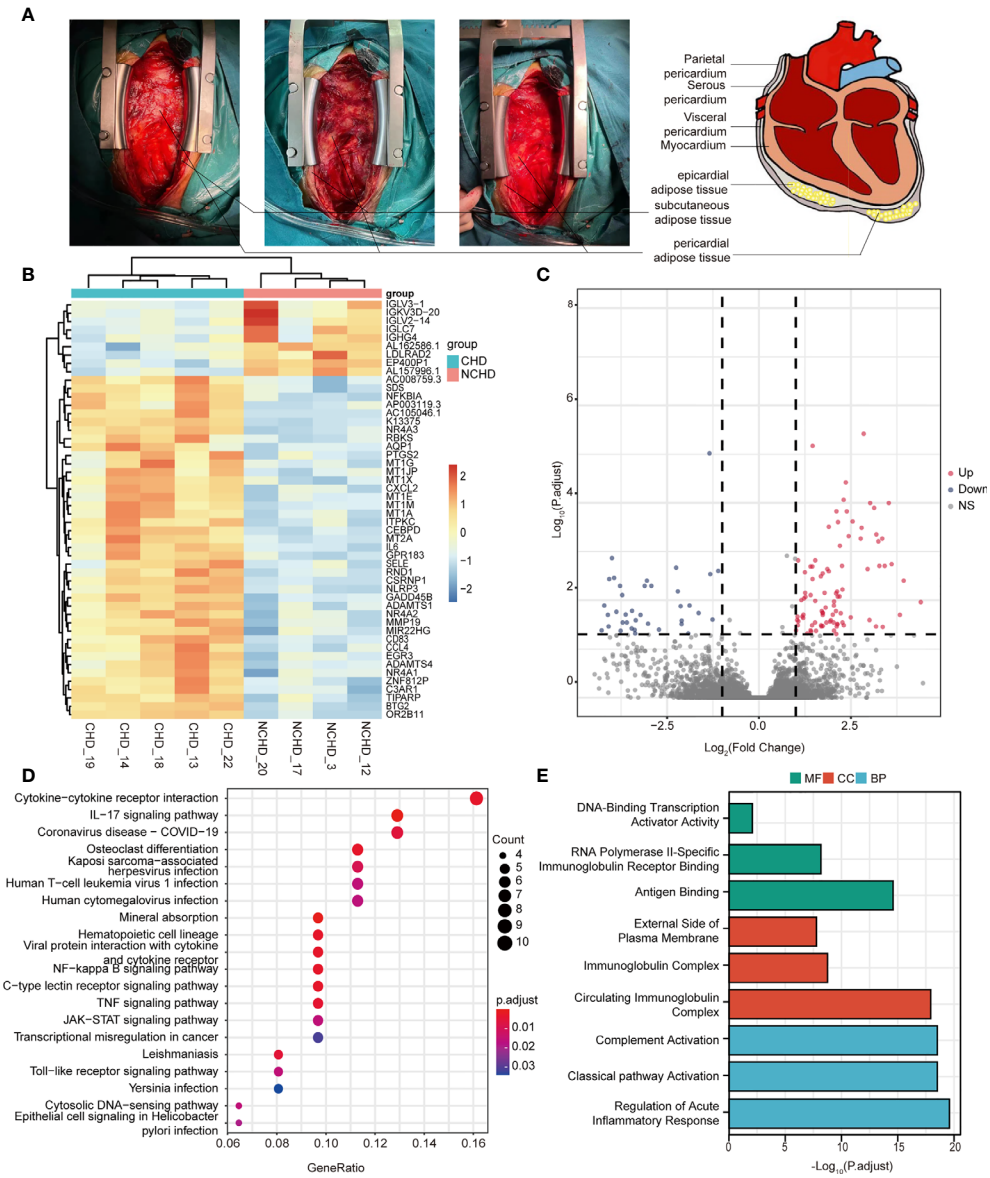

FIGURE 1 | **(A)** Localization of pericardial fat; **(B)** Heatmap results of DEGs; **(C)** Volcano plot results of DEGs; **(D, E)** The significantly enriched KEGG and GO terms that correspond to coding gene functions of upregulated and downregulated DEGs.

mRNA Isolation and Real-Time PCR

Total RNA was isolated from adipose tissue biopsies using an RNA rapid extraction kit (TR205-200, Tianmo biotech, Beijing, China) following the manufacturer's protocol. RT-PCR was performed using a cDNA reverse transcription kit (RR047A, TAKARA, Japan), Premix Ex Taq (RR420A, TAKARA, Japan) in 7500 Real-Time PCR system (Applied Biosystems, San Francisco, CA, USA). Standard and melting curves were generated in every plate for each gene to ensure that the reaction is efficient and specific. The cycle threshold value of β-actin acted as the internal control. The relative expression levels of different genes were analyzed *via* the 2−ΔΔCT method. Primer sequences were obtained from PrimerBank (http://pga.mgh.harvard.edu/ primerbank) as follows (**Table 1**): JUN forward TCCAAGTGCCGAAAAAGGAAG, JUN reverse CGAGTTCTGAGCTTTCAAGGT; ATF3 forward CGAGTTCTGAGCTTTCAAGGT, ATF3 reverse TTCTTTCTCGTCGCCTCTTTTT; CXCR4 forward ACTACACCGAGGAAATGGGCT, CXCR4 reverse CCCACAATGCCAGTTAAGAAGA; FOSB forward GCTGCAAGATCCCCTACGAAG, FOSB reverse ACGAAGAAGTGTACGAAGGGTT; CCL4 forward CTGTGCTGATCCCAGTGAATC, CCL4 reverse TCAGTTCAGTTCCAGGTCATACA; CXCL2 forward

TABLE 1 | The primer sequences about hub genes.

Gene symbal	Forward Primer	Reverse Primer
JUN	TCCAAGTGCCGAAAAAGGAAG	CGAGTTCTGAGCTTTCAAGGT
ATF3	CGAGTTCTGAGCTTTCAAGGT	TTCTTTCTCGTCGCCTCTTTTT
CXCR4	ACTACACCGAGGAAATGGGCT	CCCACAATGCCAGTTAAGAAGA
FOSB	GCTGCAAGATCCCCTACGAAG	ACGAAGAAGTGTACGAAGGGTT
CCL4	CTGTGCTGATCCCAGTGAATC	TCAGTTCAGTTCCAGGTCATACA
CXCL2	CTCAAGAATGGGCAGAAAGC	CTCCTAAGTGATGCTCAAAC

CTCAAGAATGGGCAGAAAGC, CXCL2 reverse CTCCTAAGTGATGCTCAAAC (17).

RNA Sequencing

Paired-end libraries were synthesized using the TruSeq™ RNA Sample Preparation Kit (Illumina, USA) following the TruSeq™ RNA sample preparation guide. Briefly, the poly-A containing mRNA molecules were purified using poly-T oligo-attached magnetic beads. Thereafter, the mRNA was fragmented into small pieces using divalent cations under 94°C for 8 min. The cleaved RNA fragments were copied into first-strand cDNA using reverse transcriptase and random primers. Subsequently, the second strand cDNA was synthesized using DNA Polymerase I and RNase H. These cDNA fragments then underwent an end repair process, the addition of a single 'A' base, and ligation of the adapters. The products were purified and enriched with PCR to generate the final cDNA library. Clean libraries were quantified using a Qubit® 2.0 Fluorometer (Life Technologies, USA), and validated by Agilent 2100 bioanalyzer (Agilent Technologies, USA) to confirm the insert size and evaluate the mole concentration. Cluster was generated using cBot with the library diluted to 10 pM and then was sequenced on the Illumina NovaSeq 6000 (Illumina, USA). Library construction and sequencing were performed by Sinotech Genomics Co., Ltd (Shanghai, China).

Data Processing of DEGs

Gene abundance was expressed as fragments per kilobase of exon per million reads mapped (FPKM). We employed the Stringtie software to count the fragments within each gene. TMM algorithm was applied for normalization. The DEGs between CAD and control patients were detected in the Illumina data collection software, whereby the P-value and $|\log_2 FC|$ were calculated. Genes that met the cutoff criteria, P value<0.05 and $|\log_2 FC|>1.0$, considered as significantly modulated, were retained for subsequent analysis.

Enrichment Analysis

The Gene Ontology (GO) analysis and a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis were performed in the R package cluster Profiler (version 3.18.0) (18). GO enrichment analysis included biological processes (BP), cellular components (CC), and molecular functions (MF).

PPI Network Construction, Significant Module, and Hub Genes Analysis

The PPI network was first analyzed using Cytoscape (version 3.7.2, www.cytoscape.org) software after which the key genes in the PPI networks were identified using cytohubba (version 1.4.2), a plug-in of the Cytoscape software (19, 20). The Molecular Complex Detection tool (MCODE) (version 1.5.1) and Metascape (http://metascape.org/gp/) were employed to screen the significant module (21, 22).

Identification and Analysis of Significant Genes

A Venn diagram was delineated to identify significant common genes across "Metascape_MCODE", "Cytoscape_MCODE", and "Cytoscape_cytoHubba" by Veeny2.1 (https://bioinfogp.cnb.csic.es/tools/venny/). Summarized functions of significant genes were obtained *via* GeneCards (https://www.genecards.org/) (23).

Analyses of miRNA-mRNA Targets

We applied the miRNA-target tool ENCORI to predict the miRNA of DEGs (24). There were nine databases about miRNA-mRNA prediction. miRNAs predicted in at least two databases were selected as the potential target miRNAs of hubgenes. Then, the Cytoscape software was employed to assess the regulatory networks of the miRNA-mRNA pairs.

Immune Cell Infiltration

The immune cell components in adipose tissue were analyzed *via* CIBERSORT (25).

Prediction of Drugs Targeting DEGs

The Drug–Gene Interaction Database (DGIdb, www.dgidb.org) is a web resource that organizes and presents gene druggability information and drug-gene interactions from databases, articles, and web resources (26). Herein, we used DGIdb (version 3.0.2) to predict the potential drugs that target key DEGs confirmed *via* network module analysis. The following parameters were used: Preset filter, Food, and Drug Administration approved; advanced filters, source databases: all; gene categories, all; and interaction types, all. The interaction network was constructed using Cytoscape.

Statistics

Comparison of the clinical characteristics was achieved using the 2-tailed Student's t-test for continuous variables or the $\chi 2$ test for dichotomous variables. A p-value is less than 0.05 denoted significance. Adipocyte size and positive cell numbers were compared *via* a 2-tailed Student's t-test. For the qRT-PCR experiment, expression values relative to β-actin were compared using 2-way ANOVA and Tukey's multiple comparisons test.

RESULTS

Clinical Characteristics of Subjects

Table 2 outlines the clinical characteristics of 31 subjects. The mean age of the CAD group and control group were 66.06 ± 7.94 years and 60.93 ± 12.03 years, respectively (P = 0.165). Except for a trend towards more female patients in the control group (17.6% *vs* 42.9%, P = 0.124) and diabetes history in the CAD group (7/17 *vs* 2/14, P=0.101), we reported no difference in age, gender, BMI, comorbid conditions, and clinical biochemical characteristics. As such, our study groups were well matched. The clinical features of 9 cases who underwent RNA sequencing are outlined in **Supplemental Table 1**. Discriminant multivariate analysis demonstrated that the two groups were well matched.

Depot-Specific Transcriptomic Profiles of PAT in CAD and Control Groups

RNA-seq was performed in 9 patients (CAD=5, Control=4), we first sought to identify depot-specific gene signatures in a pairwise manner. Using threshold criteria of fold change (FC) greater than or equal to 2 or less than or equal to –2 and p-value less than or equal to 0.05, 147 differentially expressed genes (DEGs) (104 upregulated and 43 downregulated) were identified in the CAD group relative to the control group (i.e., CAD vs. control groups) (**Figures 1B, C**). In addition, the top significantly changing genes were IL6, FOSB, CSF3, IGLC7, and PPP1R14C; these genes were associated with inflammation, nuclear transcription, cell differentiation, immunity, and neuronal activity.

Function and Pathway Enrichment Analyses

To explore the relative functions and pathways of DEGs in CAD *vs*. control groups, we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis on those DEGs. The DEGs were significantly enriched in regulation of acute inflammatory response, regulation of complement activation, and complement activation classical pathway associated biological process (BP) terms, circulating immunoglobulin complex, immunoglobulin complex, and external side of plasma membrane associated component cell (CC) terms, antigen binding, RNA polymerase II-specific immunoglobulin receptor binding, and DNA-binding transcription activator activity associated molecule function (MF) terms (**Figure 1E**). KEGG pathway analysis demonstrated the enrichment of DEGs in cytokine-cytokine receptor interaction, IL-17 signaling pathway, and COVID-19 (**Figure 1D**).

PPI Network Construction and Module Analysis of DEGs

We systematically analyzed the biological functions of the obtained DEGs between the two groups using a PPI network of DEGs constructed *via* the STRING database and visualized using Cytoscape. The PPI network comprised 77 nodes (proteins) and 271 edges (interactions; **Figure 2A**). The 77 nodes included 71 up-regulated genes and 6 down-regulated genes. According to the topology score, Interleukin-6 (IL-6), Jun proto-oncogene (JUN), and prostaglandin-endoperoxide synthase 2 (PTGS2) activating transcription factor 3 (ATF3), chemokine (C-X-C motif) receptor 4 (CXCR4) were the top five genes (**Figure 2B**). Additionally, five MCODE modules were identified from the PPI network *via* cytoscape_CMODE; however, one submodule with a score >5 was extracted from the PPI network, which comprised 8 nodes and 26 edges (**Figure 2C**). In this module, IL-6, chemokine (C-X-C motif) ligand 2 (CXCL2), selectin E (SELE), C-C motif chemokine ligand 4 (CCL4), PTGS2 were the top five genes (**Figure 2C**). Three MCODE modules were extracted through Metascape analysis (**Figure 2D**).

TABLE 2 | Clinical characteristics of the subjects.

Parameters	CAD (n=17)	NCAD (n=14)	p value
Sex (male/female)	14/3	8/6	0.124
Age (years), mean±SD	66.06 ± 7.94	60.93 ±12.03	0.165
BMI (kg/m2), mean±SD	24.7 ± 3.48	25.69 ± 4.26	0.489
Hypertension (Yes/No)	10/7	7/7	0.623
Diabetes (Yes/No)	7/10	2/12	0.101
Stroke (Yes/No)	4/13	1/13	0.217
Smoking (Yes/No)	4/13	1/13	0.217
Total-cholesterol (mM), mean±SD	4.15 ± 1.19	4.55 ± 1.12	0.350
Triglycerides (mM), mean±SD	1.90 ± 1.36	1.50 ± 0.58	0.293
HDL-cholesterol (mM), mean±SD	1.10 ± 0.18	1.31 ± 0.42	0.200
LDL-cholesterol (mM), mean±SD	2.62 ± 1.24	2.45 ± 0.76	0.725
ESR (mm/h), mean±SD	13.44 ± 12.39	14.31 ± 6.77	0.835
CRP (mg/L), mean±SD	10.33 ± 14.55	6.49 ± 5.64	0.368
WBC (10^9/L), mean±SD	6.71 ± 1.39	6.41 ± 2.31	0.662
N (%), mean±SD	66.06 ± 10.09	66.53 ± 13.42	0.913
Hb (g/L), mean±SD	135.88 ± 14.73	132.57 ± 21.58	0.617
ALT (mM), mean±SD	29.61 ± 22.70	29.10 ± 15.10	0.944
AST (mM), mean±SD	25.91 ± 12.02	24.91 ±9.77	0.805
BUN (mM), mean±SD	6.36 ± 1.64	5.84 ± 2.13	0.446
CR (mM), mean±SD	79.04 ± 15.47	81.99 ± 30.72	0.748
eGFR, mean±SD	88.69 ± 14.90	84.67 ± 23.50	0.579
UA (mM), mean±SD	364.46 ± 93.92	386.91 ± 137.50	0.594

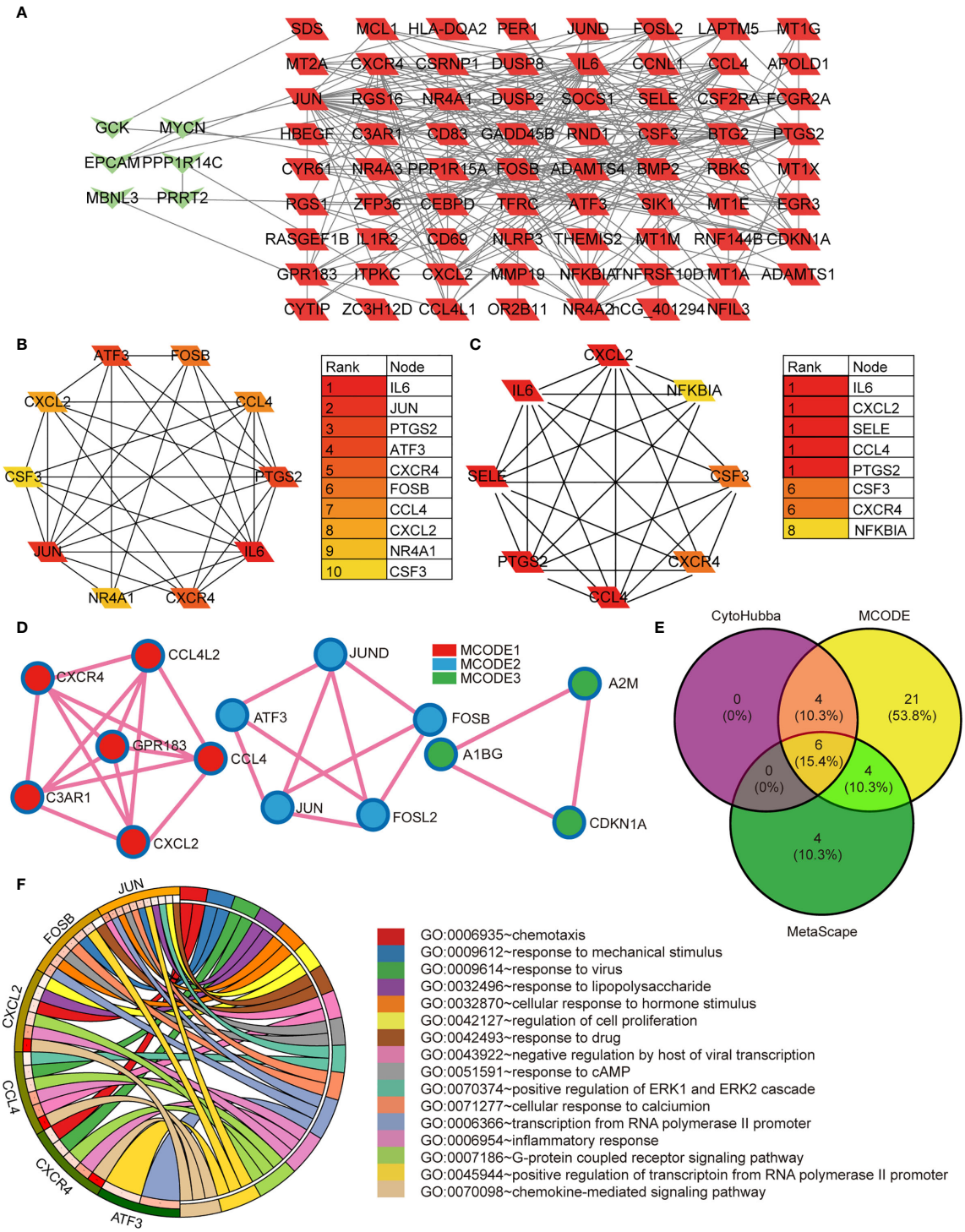

FIGURE 2 | **(A)** The protein-protein interaction network constructed based on the DEGs. The green heart represents the downregulated genes, the red parallelogram represents the upregulated genes. **(B–D)** The hubgenes predicted by cytohubba, Mcode, and metascape Mcode, respectively. **(E)** Overlap of hubgenes identified *via* the three methods; **(F)** The Pathway analysis results showing the six enriched hubgenes.

Identification and Analysis of Significant Genes

To reveal the most important hub gene, we filtered these genes using the VENN diagram. The VENN diagram revealed six significant common genes, including JUN, ATF3, CXCR4, FOSB, CCL4, and CXCL2 (**Figure 2E**). The functions of the six significant genes are summarized in **Table 3**. We shifted our focus to explore the potential function of these six key genes. Pathway analysis demonstrated that the six genes were mainly enriched in the chemokine-mediated signaling pathway, inflammatory response, transcription from RNA polymerase II promoter, G-protein coupled receptor signaling pathway, and positive regulation of transcription from RNA polymerase II promoter (**Figure 2F**). RT-qPCR analysis revealed that the relative expression levels of JUN (**Figure 3A**), FOSB (**Figure 3B**), ATF3 (**Figure 3C**), CCL4 (**Figure 3D**), and CXCR4 (**Figure 3E**) were significantly higher in the CAD group adipose tissue than in control group.

miRNA-mRNA Interaction Network

We explored the effect of PAT on CAD progression and its potential gene regulation mechanism. miRNA-target gene interaction pairs of reverse association were predicted *via* ENCORI according to the hub genes identified previously. Considering the identified miRNA-mRNA pairs, we compared the interaction network containing 70 miRNA-mRNA pairs and visualized them *via* the Cytoscape software. Through comparison of the targets of hub genes, we found CXCR4 to be a potential target of 23 miRNAs, including hsa-miR-185-5p. Also, FUN and FOSB were the potential targets of 15 miRNAs. The miRNA-gene regulation network is illustrated in **Table 4** and **Figure 4**, respectively.

Macrophages Are the Major Immune Infiltrating Cell Subset

GO and KEGG enrichment analysis has shown inflammatory response is the major procession in PAT of CAD patients. However, the component of immune cells in the pericardial adipose deposit is unknown. We employed CIBERSORT, a bioinformatics tool used to infer immune cell composition from RNA-seq datasets, to compute the relative frequency of 22 infiltrating immune cell subsets in the 9 cases. Results revealed that monocytes and macrophages were the most common immune cell subsets with mean fractions of 0.098 and 0.325, respectively (**Figure 5**). Further, we verified the inflammatory phenotype of human PAT from both CAD and control patients through immunohistochemistry analysis of markers of T cell (CD3), macrophage (CD68), and its M1 subtype (CD11b). We found that PAT from CAD patients had significantly more T cells (CD3+) and macrophages (CD68+) than that of control patients (**Figure 6**). Semi-quantification analysis revealed that the IOD/Area of macrophages and T-cells in PAT of CAD patients was significantly different (**Table 5**). These findings further proved that PAT exerts a potential inflammatory effect in the regulation of CAD.

Drug Predictable Results

The search for potential drugs that can improve the function of PAT to intervene in the disease process of CAD propelled us to analyze the six key genes for potential drugs *via* DGIdb. Finally, five genes were considered as druggable genes, including JUN, CXCL2, and CXCR4; also we obtained 77 possible drugs. An interaction network including 82 nodes and 77 edges was constructed based on the five druggable genes (**Figure 7**).

DISCUSSION

In this study, we have sequenced the transcriptome of PAT from five patients with CAD and four patients without CAD and analyzed the DEGs between two groups. A total of 147 DEGs (104 up-regulated genes and 43 down-regulated genes) were identified. We subjected the DEGs to GO functional and KEGG pathway enrichment analyses *via* Clusterprofile package, and obtained 40 significant GO terms and 55 significant KEGG pathways. GO term also demonstrated the interaction of these genes with immune and inflammatory cells, and we, therefore, employed the Cibersort online tool (https://cibersort.stanford.edu/) to analyze 22 types of immune cells infiltration. Based on the results, monocytes and macrophages were the most common immune cell subsets. Further immunohistochemical staining demonstrated that more macrophages were present in PAT from CAD patients, and the proportion of M1 subtype cells was higher. These findings imply that PAT inflammation potentially plays a crucial role in CAD progression.

Moreover, GO and KEGG pathway analysis results revealed the significant enrichment of DEGs in cytokine-cytokine receptor interaction, IL-17 signaling pathway, and COVID-19,

TABLE 3 | The enrichment results of GO and KEGG of hubgenes.

Category	Term	P value
GO	chemokine activity	0.000104
	chemokine receptor binding	0.000213
	cytokine activity	0.002367
	cytokine receptor binding	0.003134
	G protein-coupled receptor binding	0.003652
KEGG	IL-17 signaling pathway	1.49E-05
	Viral protein interaction with cytokine and cytokine receptor	1.79E-05
	Chemokine signaling pathway	0.000126
	Cocaine addiction	0.000354
	Cytokine-cytokine receptor interaction	0.000452

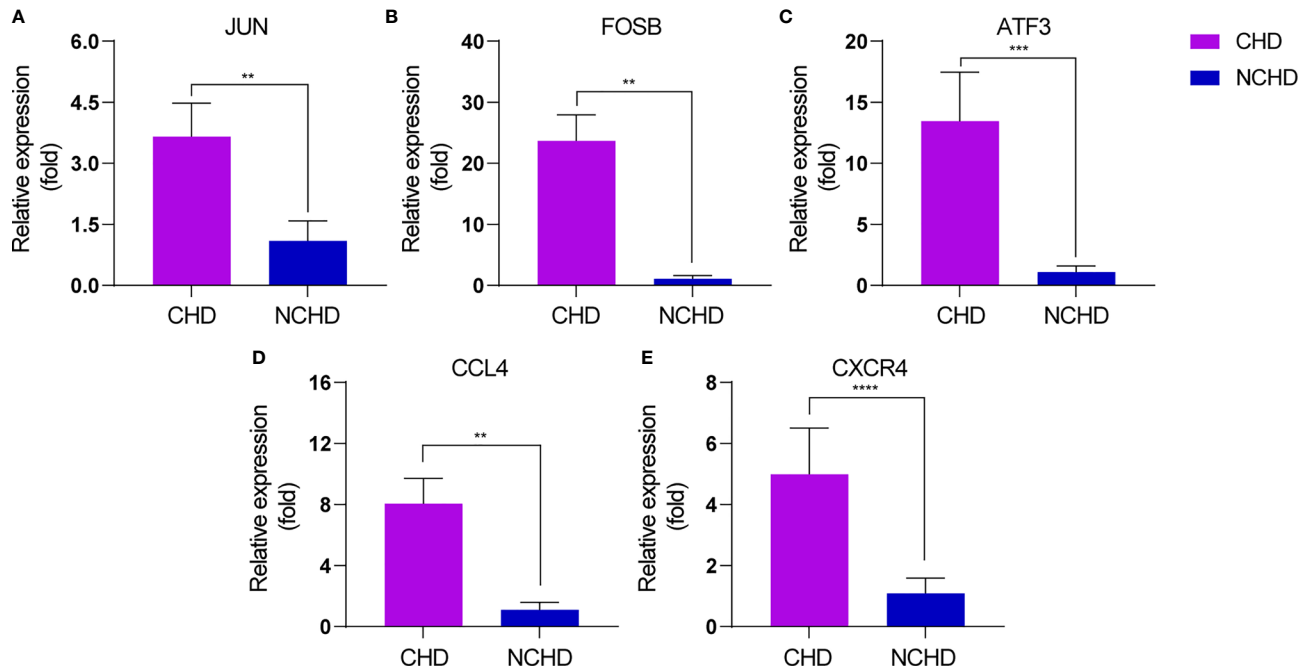

FIGURE 3 | **(A–E)** RT-PCR verification of the expression levels of 5 genes (JUN, FOSB, ATF3, CCL4, CXCR4). The 2−ΔΔCT method was used to analyze the relative expression levels of various genes. * means 0.05 < p<0.1; ** means p < 0.05; *** means p < 0.01; **** means p < 0.001.

TABLE 4 | The predicted miRNA about 6 hubgenes.

Gene symbol	Count	miRNA		
JUN	15	hsa-miR-92b-3p	hsa-miR-524-5p	hsa-miR-200c-3p
		hsa-miR-758-3p	hsa-miR-522-3p	hsa-miR-200b-3p
		hsa-miR-5688	hsa-miR-495-3p	hsa-miR-200a-3p
		hsa-miR-542-3p	hsa-miR-429	hsa-miR-141-3p
		hsa-miR-216b-5p	hsa-miR-340-5p	hsa-miR-139-5p
ATF3	9	hsa-miR-1224-5p	hsa-miR-135b-5p	hsa-miR-224-5p
		hsa-miR-135a-5p	hsa-miR-155-5p	hsa-miR-27a-3p
		hsa-miR-27b-3p	hsa-miR-513a-5p	hsa-miR-7-5p
CXCR4	24	hsa-miR-9-5p	hsa-miR-494-3p	hsa-miR-302d-3p
		hsa-miR-655-3p	hsa-miR-4306	hsa-miR-302c-3p
		hsa-miR-613	hsa-miR-410-3p	hsa-miR-302b-3p
		hsa-miR-588	hsa-miR-374c-5p	hsa-miR-302a-3p
		hsa-miR-520e	hsa-miR-338-3p	hsa-miR-300
		hsa-miR-204-5p	hsa-miR-302e	hsa-miR-211-5p
		hsa-miR-185-5p	hsa-miR-1-3p	hsa-miR-206
		hsa-miR-139-5p	hsa-miR-27a-4p	
FOSB	15	hsa-miR-613	hsa-miR-27a-3p	hsa-miR-200a-3p
		hsa-miR-342-3p	hsa-miR-23b-3p	hsa-miR-185-5p
		hsa-miR-27b-3p	hsa-miR-23a-3p	hsa-miR-182-5p
		hsa-miR-141-3p	hsa-miR-130a-5p	hsa-miR-144-3p
		hsa-miR-1-3p	hsa-miR-128-3p	hsa-miR-212-5p
CCL4	8	hsa-miR-620	hsa-miR-4784	hsa-miR-3150b-3p
		hsa-miR-496	hsa-miR-324-5p	hsa-miR-185-5p
		hsa-miR-143-3p	hsa-miR-1270	
CXCL2	12	hsa-miR-641	hsa-miR-532-5p	hsa-miR-217
		hsa-miR-582-5p	hsa-miR-495-3p	hsa-miR-215-5p
		hsa-miR-5688	hsa-miR-376c-3p	hsa-miR-193b-3p
		hsa-miR-193a-3p	hsa-miR-192-5p	hsa-miR-128-3p

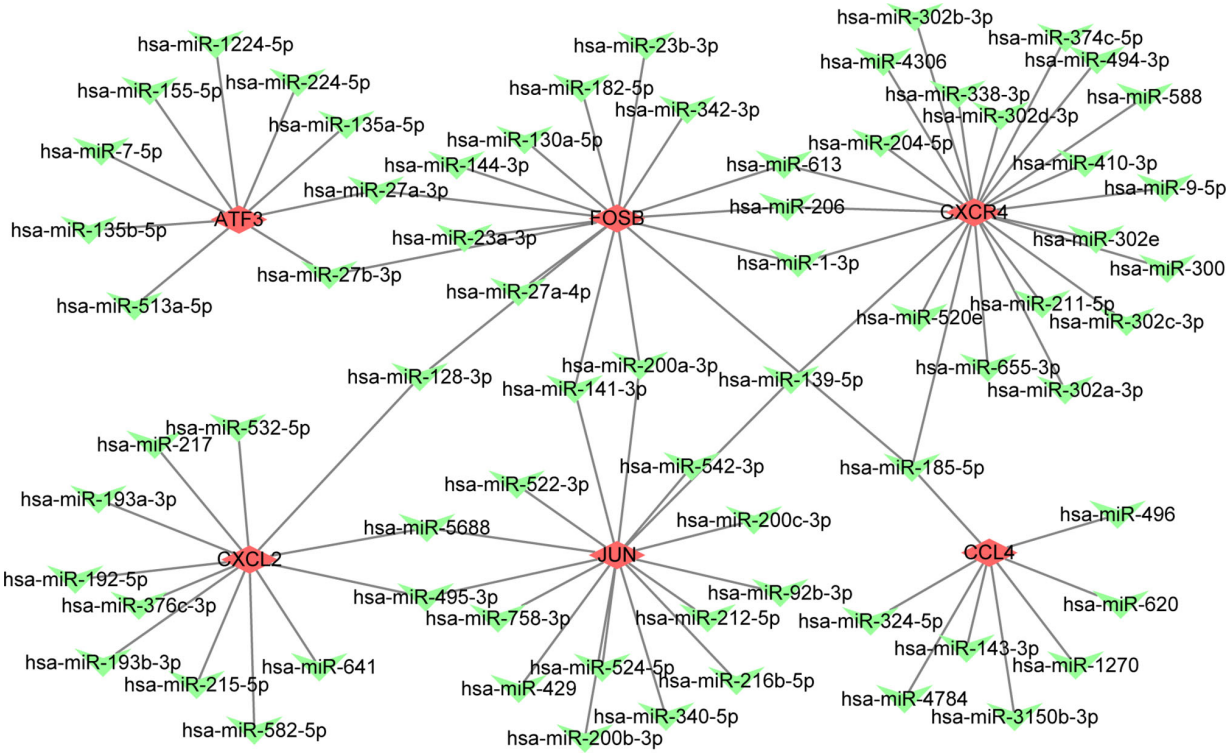

FIGURE 4 | The constructed mRNA-miRNA network. The green heart represents the downregulated genes, the red diamond represents the hubgenes.

all of which are inflammatory processes. Compelling evidence implicates the IL-17 signaling pathway in the pathogenesis of many inflammatory-driven conditions, including atherosclerosis (27–30). A study found that IL-17 maintained plaque stability through induction of proliferation of SMCs and collagen content in atherosclerotic plaques (29). Also, IL-17 had been revealed to potentially lower the expression of vascular cell adhesion molecule (VCAM)-1 in endothelial cells and prevent monocyte adherence and block T cell infiltration into plaques (31). Other reports show that IL-17 can induce the release of chemokines, including CXCL1, CXCL2, CXCL8, CXCL10. Subsequently, these chemokines can recruit neutrophils and monocytes to the atherosclerotic lesion (32, 33). Furthermore, IL-17 can stimulate macrophages to produce inflammatory cytokines, such as IL-6, TNF-a, and IL-1b (34–36), and induce apoptosis of vascular endothelial cells through activation of caspase-3 and caspase-9. These observations affirm that the IL-17 signaling pathway mediates atherosclerosis (37). We also found that other signaling pathways such as NF-kappa B signaling pathway, TNF signaling pathway, JAK-STAT signaling pathway, and Toll-like receptor signaling pathway were enriched in PAT of CAD patients. Of note, some proteins in these pathways can interplay with each other, thereby increasing the severity of inflammation and apoptosis (38). These results imply that the management of inflammation in CAD may require nonspecific inflammatory inhibitors.

The PPI network-integrated three modules in the analysis of six significant and reproducible genes (JUN, ATF3, CXCR4, FOSB, CCL4, CXCL2), and revealed differential expression between the CAD and control groups. Three core genes (CXCR4, CXCL2, and CCL4) were markedly enriched in the chemokine signaling pathway, whereas the others (Jun, FosB, and ATF3) were transcription factors. Chemokines are cytokines that mediate cell chemotaxis and stagnation as they bind to their respective cell surface receptors (39). Chemokines induce the aggregation of inflammatory cells to the inflammatory site, which is the main factor that trigger vascular inflammatory injury (39, 40). Previous reports indicate that CCL4 is highly expressed in atherosclerotic patients (41), and it potentially exerts a crucial role in the progression of atherosclerotic plaque (42–44). In addition, inhibition of CCL4 can stabilize atherosclerotic plaques by decreasing the expression of MMPs, inflammatory cell infiltration, circulation of inflammatory factors, and regulation of blood lipid levels (43). The C-X-C motif chemokine receptor 4 (CXCR4) is a cytokine receptor and mediates various biological processes (45). The ligand of CXCR4 is C-X-C motif chemokine ligand 12 (CXCL12) (46). CXCL12 and CXCR4 coordinatively play a pivotal role in atherosclerosis and arterial injury (47, 48). CXCR4 expression in leukocytes is closely associated with the vulnerability of atherosclerosis plaque (49). A recent study found that CXCR4-positive macrophages accumulated in tissue samples of human carotid plaques and,

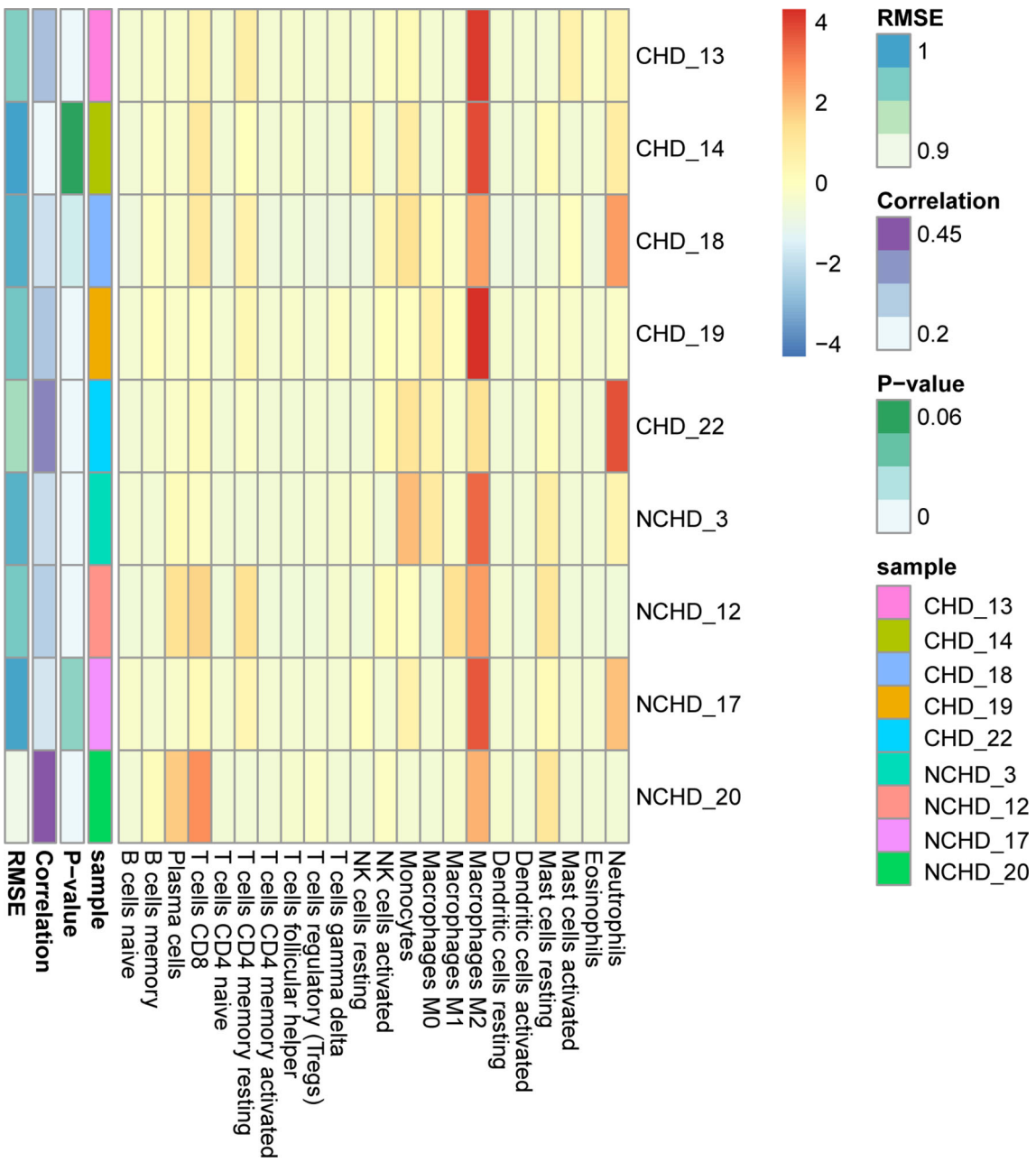

FIGURE 5 | Distinction of infiltrating immune cell subpopulations and levels between CHD/NCHD groups.

CXCR4 was expressed in both smooth muscle cell progenitors and endothelial progenitor cells in atherosclerotic plaque progression (46). The study by Puca AA showed that upregulation of CXCR4 decreases the development of atherogenic process. This is because it can skew macrophages to acquire an M2-resolving phenotype, maintains maintain arterial integrity, preserves preserve endothelial cell integrity, and restore the normal contractile SMC phenotype (50, 51). In contrast to CXCR4, CCL4 inhibition reduced the adhesiveness of coronary endothelial cells, which is an early sign of atherogenesis (43). Herein, we found that CCL4, CXCR4 derived from PAT was highly expressed at the mRNA level in CAD patients. More exploration of CCL4 and CXCR4 secreted by PAT would enhance the understanding of the pathophysiological mechanism of atherosclerosis and facilitate its utilization as a biomarker and intervention target for atherosclerosis.

Activator protein-1 (AP-1) is an important class of nuclear transcription factors in the body. It is a homodimer or heterodimer composed of JUN, FOS, ATF, and MAF protein families which exert biological effects (52). AP-1 is largely

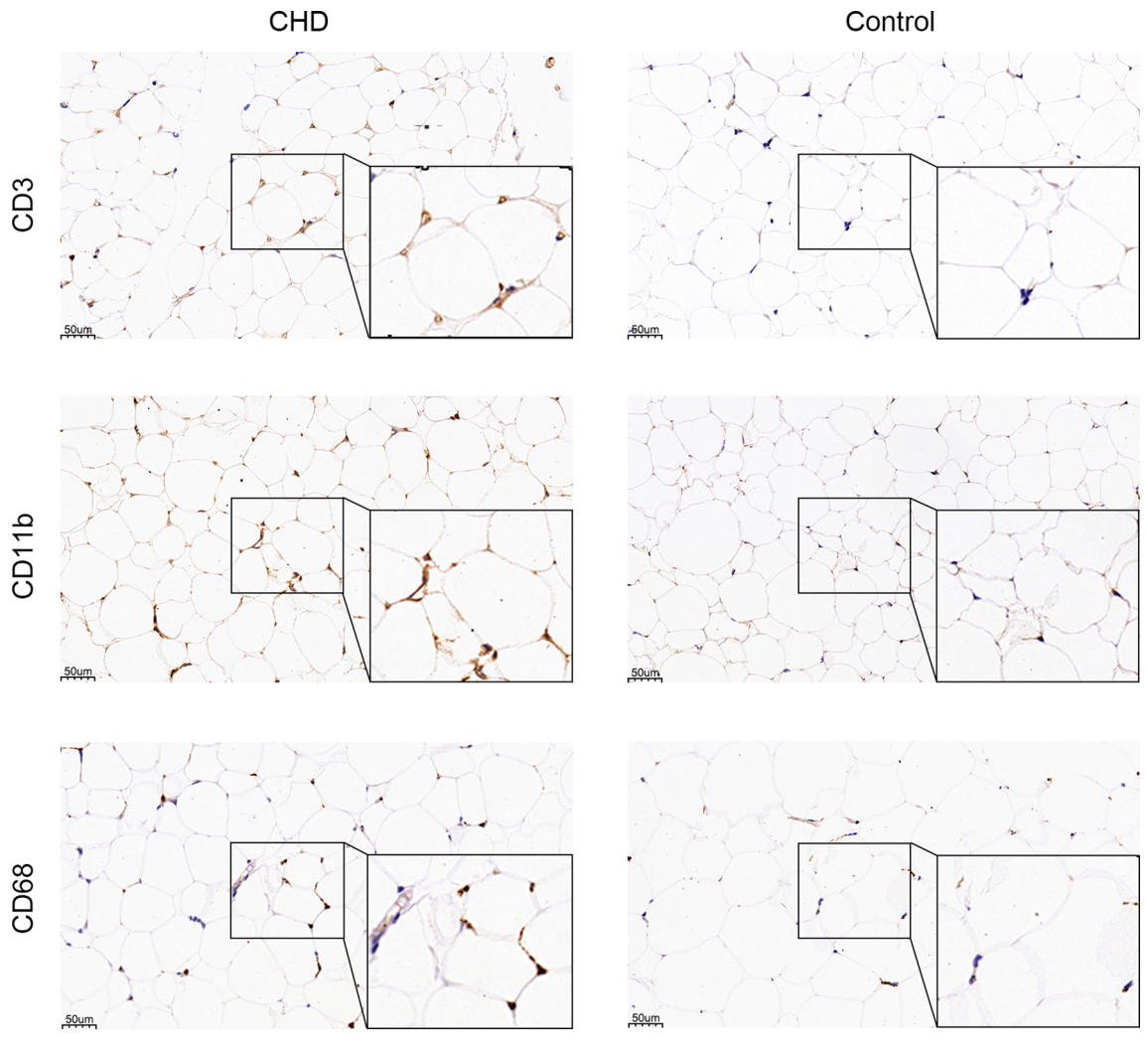

FIGURE 6 | IHC staining of CD68, CD11b, and CD3. CD68, CD11b, and CD3 protein are stained in brown.

associated with cell proliferation, differentiation, apoptosis, and inflammation (53–55). Previously, the AP-1 cascade was found to potentially drive additional leukocyte recruitment to the developing atherosclerotic matrix (56). AP-1 could also promote the development of atherosclerosis by inducing endothelial cell death, the proliferation and migration of vascular smooth muscle cells (57, 58). In this study, we observed a significant increase in mRNA expression of AP-1 factors JUN, FOSB, and ATF3 from PAT in CAD patients. These results imply that PAT-derived AP-1 has potential effects on the progression of the coronary artery through cell proliferation, inflammation, and extracellular matrix remodeling.

Based on bioinformatics, we constructed the miRNA-mRNA network of core genes through the ENCORI database to further elucidate the regulatory mechanism of core genes. Among the predicted miRNAs, only miRNA-185-5p bound to three of the six core genes (FOSB, CCL4, and CXCR4). Previous studies had confirmed that miRNA-185-5p is widely involved in the proliferation, metastasis, and inflammation of various tumors (59–61). However, its regulatory role in inflammation and the progression of CAD remains elusive.

Through the prediction of the Drug-Gene Interaction database, we revealed some drugs that potentially interact with the core genes. Some drugs were used in the clinic but not to manage CAD. For instance, colchicine, an anti-inflammatory alkaloid, has been mainly used in gout diseases (62); it exerts inhibitory effects on leukocyte chemotaxis and adhesion, microtubule assembly, and reduces the

TABLE 5 | The semi-quantification analysis of infiltrating immune cell.

Cell type	CHD	Contral
T cells (IOD/Area)	0.093	0.075
Macrophages (IOD/Area)	0.337	0.177

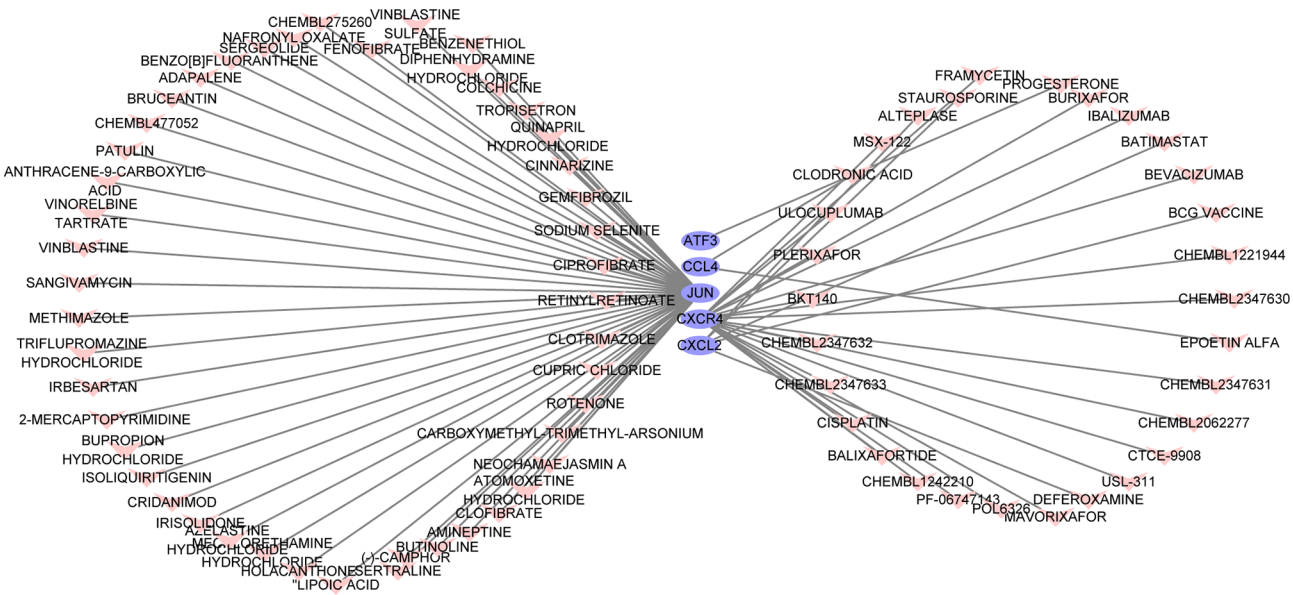

FIGURE 7 | The constructed mRNA-drug network. The purple oval represents the genes, and the light red heart represents the drugs.

production of inflammatory mediators (63, 64). Because of its anti-inflammatory properties, the feasibility of the value of colchicine in CAD had been investigated in several clinical trials. Most clinical trials suggest that colchicine is beneficial in patients with CAD or myocardial infarction (65–67). However, some studies revealed that colchicine could reduce major cardiovascular events (68), however, the overall mortality did not decrease. As such, colchicine may increase the mortality of non-cardiovascular events and limit its application in CAD (69), which warrants more large-scale clinical trials. Fenofibrate is a broad-spectrum lipid-lowering drug, which acts on PPAR to potentially reduce cholesterol and triglyceride (70, 71). Elsewhere, a study found that fibrates have potential anti-inflammatory effects, protect endothelial cell function, and improve insulin resistance (72). However, these drugs are not recommended for the secondary prevention of CAD. In clinical trials such as HHS, VA-HIT, and BIP, fibrates have proved to exhibit significant benefits in subgroups of patients, including those with insulin resistance or metabolic syndrome (73–75). Recent studies have further outlined that fibrates can reduce the risk of cardiovascular events and the level of uric acid in diabetic patients with dyslipidemia (76, 77). Collectively, fibrates may have great clinical prospects in some specific groups of patients with CAD.

In summary, this work has allowed for the identification of the transcriptome characteristic shift in PAT from humans with or without CAD and revealed the upregulation in inflammatory processes of PAT in CAD patients. The crucial genes, pathways, and drug target genes closely associated with CAD have been revealed through bioinformatics analyses. Particularly, the critical genes are potential biomarkers and therapeutic targets in CAD. Also, colchicine and fenofibrate are the most promising drugs for coronary artery disease.

LIMITATIONS OF THE STUDY

The primary limitation of the research is that we did not analyze samples from healthy subjects; this could have been more interesting for the PAT physiology study. Besides, the analyzed samples from older patients with some comorbidity who came in for various cardiac surgeries rather than no comorbidity. We also acknowledge that we had a rather small RNA-Seq sample size, which could not allow us to analyze samples grouped by obesity, age, and gender. This may have contributed to the CAD-associated transcriptomic shifts.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in GEO (Gene Expression Ominbus), a public functional genomics data repository. The accession number(s) in the GEO can be found below: GSE179397.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of Huadong Hospital Affiliated to Fudan University, Shanghai, China. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ML and LQ have contributed equally to this work and share first authorship. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.724859/full#supplementary-material

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REVIEW
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The Sick Adipose Tissue: New Insights Into Defective Signaling and Crosstalk With the Myocardium

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Adipose tissue (AT) biology is linked to cardiovascular health since obesity is associated with cardiovascular disease (CVD) and positively correlated with excessive visceral fat accumulation. AT signaling to myocardial cells through soluble factors known as adipokines, cardiokines, branched-chain amino acids and small molecules like microRNAs, undoubtedly influence myocardial cells and AT function *via* the endocrine-paracrine mechanisms of action. Unfortunately, abnormal total and visceral adiposity can alter this harmonious signaling network, resulting in tissue hypoxia and monocyte/macrophage adipose infiltration occurring alongside expanded intra-abdominal and epicardial fat depots seen in the human obese phenotype. These processes promote an abnormal adipocyte proteomic reprogramming, whereby these cells become a source of abnormal signals, affecting vascular and myocardial tissues, leading to meta-inflammation, atrial fibrillation, coronary artery disease, heart hypertrophy, heart failure and myocardial infarction. This review first discusses the pathophysiology and consequences of adipose tissue expansion, particularly their association with meta-inflammation and microbiota dysbiosis. We also explore the precise mechanisms involved in metabolic reprogramming in AT that represent plausible causative factors for CVD. Finally, we clarify how lifestyle changes could promote improvement in myocardiocyte function in the context of changes in AT proteomics and a better gut microbiome profile to develop effective, non-pharmacologic approaches to CVD.

Keywords: adipose tissue, myocardiocytes, microbiota, obesity, inflammation

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1 INTRODUCTION

Obesity is a chronic and multifactorial metabolic disease described in most scientific literature as the epidemic of the 21st century. In fact, by 2016, this condition affected 650 million adults, equivalent to 13% of the adult population worldwide, while in 2019, 38.3 million children under the age of 5 were overweight or obese (1). In the United States, obesity accounts for approximately 21% of

annual national health care costs ($190 billion) (2). In addition, this entity is frequently clustered to other comorbidities such as metabolic syndrome (MetS), insulin resistance (IR), type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD), chronic kidney disease (CKD), gout, and cardiovascular disease (CVD) (3). CVD is the leading cause of death worldwide, with approximately 17.9 million deaths each year, of which 85% are attributable to myocardial infarction (MI) and stroke (4).

Research has centered on evaluating the causality of obesity in CVD in recent years, focusing on areas such as the potential role of adipose tissue (AT) on cardiac tissue (5, 6). AT is a highly functional and complex endocrine organ, characterized by the release of adipokines, batokines, microRNAs, prostaglandins, bioactive lipids and other regulators of metabolic homeostasis, which interact with vascular, hepatic, renal, digestive, cerebral, skeletal muscle and myocardial tissue through paracrine and endocrine mechanisms (5, 7–10).

One hallmark feature in obesity is the ectopic and visceral adipose tissue (VAT) accumulation leading to AT transcriptome and secretome modification due to adipocyte hypertrophy and hyperplasia. This condition is related to tissue´s hypoxia and fibrosis, immune cell infiltration, stimulating the release of pro-inflammatory, pro-atherogenic and anti-angiogenic substances that affect AT biology and communication with other target tissues (11). In addition, myocardial cells are also affected by signaling molecules from the dysfunctional or "sick" AT (SickAT), given their link with heart hypertrophy and fibrosis, atrial fibrillation (AF), MI, among other CVD (12–15).

These data highlight the importance of establishing therapeutic tools to help combat obesity and, by extension, CVD. In a nutshell, obesity etiology is derived from an energy imbalance produced in the context of an obesogenic lifestyle (16) characterized by a hypercaloric diet and insufficient physical activity (PA) to counteract the SickAT expansion and subsequent defective signaling processes (10, 17, 18). Hence, PA and nutritional interventions (NI) might improve the SickAT profile and, consequently, enhance adipose tissue and myocardiocyte crosstalk. Therefore, this review discusses both AT and SickAT distribution and biology and their relationship with myocardial tissue. We will also address the molecular mechanisms by which exercise, food supplementation, and changes in eating habits can counteract obesity, taking as a pivotal point the role of the gut microbiota (GM) in SickAT pathogenesis to establish the non-pharmacological treatment of CVD.

Abbreviations: AF, atrial fibrillation; AMI, acute myocardial infarction; AT, adipose tissue; BAT, brown adipose tissue; BeAT, beige or brite adipose tissue; BMI, body mass index; BCAAs, Branched-chain amino acids; BW body weight; C/EBP, CCAAT-enhancer-binding proteins; CKD, chronic kidney disease; CVD, cardiovascular disease; CVS, cardiovascular system; EAT, epicardial adipose tissue; eNOS, endothelial nitric oxide synthase; FFA, free fatty acid; GD, gut dysbiosis; GM, gut microbiota; HCD, hypercaloric diet; IF, intermittent fasting; iNOS, inducible nitric oxide synthase; IR, insulin resistance; LPS, lipopolysaccharides; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; NI, nutritional intervention; NP, natriuretic peptide; OS, oxidative stress; PA, physical activity; PAT, pericardial adipose tissue; PPARγ, peroxisome proliferator activated receptor γ; PVAT, perivascular adipose tissue; SickAT, sick (dysfunctional) adipose tissue; SCAT, subcutaneous adipose tissue; SCFA, short-chain fatty acids; T2DM, type 2 diabetes mellitus; WAT, white adipose tissue; VAT, visceral adipose tissue.

2 THE SICK ADIPOSE TISSUE: FROM DISTRIBUTION TO INTERACTION

The AT is a dynamic and anatomically heterogeneous organ acting as connective tissue throughout our organism. Beyond its particular vasculature, innervation and predominant adipocyte content, its microenvironment includes numerous immune cells, endothelial and stromal cells, fibroblasts, preadipocytes, and abundant extracellular matrix (ECM) (19–21). Each component possesses characteristic properties and can secrete various hormones, growth factors, microRNAs (miRNAs), cytokines, and chemokines coordinated, with autocrine, endocrine, and paracrine action on neighboring and remote organs/or cells (12, 22, 23). AT can also be classified by anatomical location, embryonic origin, morphology or function, the latter which can be grouped into white (WAT), brown adipose tissue (BAT) (24).

WAT is responsible for storing energy as fatty acids (FA) within triacylglycerides (TAG), supplying energy and controlling metabolic homeostasis through the white adipocyte endocrine functions (25). The main fat deposit in mammals is widely distributed throughout the subcutaneous adipose tissue (SCAT), gonadal and inguinal adipose depots. Adipose tissue located in the abdominal cavity, including intrahepatic and mesenteric, omental, and retroperitoneal fat, can be considered VAT (18, 19). Other intrathoracic AT depots identified include epicardial adipose tissue (EAT), occupying the space between the pericardium and myocardium, with a direct relationship with the coronary arteries; pericardial (PAT), located between the visceral and parietal pericardium, and perivascular (PVAT), which surrounds the remaining blood vessels (22, 26). It should be noted that both VAT and cardiovascular system (CVS)-based depots are considered a risk factor for cardiometabolic diseases, an association that has been widely reported (11, 26).

Unlike WAT, BAT has adipocytes with smaller lipid droplets, more abundant mitochondria and substantial vascularization, which provide its characteristic brown color (27). Likewise, BAT has high levels of uncoupling protein 1 (UCP1), which confer thermogenic properties by uncoupling between respiration and ATP synthesis during the FA oxidation in adipocytes (28, 29); hence, UCP1 is recently considered as a potential therapeutic target against obesity (30). In humans, BAT is found in specific areas (supraclavicular fossa, interscapular and paravertebral regions, in the axilla and nape) and represents only 4.3% of the total fat mass (31, 32). Notably, another type of adipocyte has been characterized within WAT deposits, and it has shown mixed characteristics of both white and brown adipocytes. For that reason, this new type of adipocyte has been coined as beige adipose tissue (BeAT). As stated above, BeAT reside within the WAT and can be mainly found within the inguinal WAT (33).

Also, BeAT express the UCP1 gene and, by extension, thermogenic properties (34). Note that this browning process occurs through exposure to cold, β-adrenergic stimulation and pharmacological modulation of WAT (35).

2.1 Changes in Adipose Tissue Microenvironment and Meta-Inflammation: The Sick Forgotten

According to the WHO, obesity is defined as excessive or abnormal fat accumulation with negative health repercussions, determined by a body mass index (BMI) ≥ of 30 kg/m^2 (36). Although its etiology includes genetic, social, environmental and/or cultural factors, in most cases, it is characterized by an imbalance between energy intake and energy expenditure, attributed to poor eating habits and sedentary lifestyles (16). This hypercaloric or overnourished state leads to more significant fat accumulation in AT, mainly in the form of ectopic or visceral depots (37). AT can increase in abundance through two different processes: hypertrophy and hyperplasia or new adipocytes formation.

Hyperplasia is considered a beneficial and adaptive process by which new functional adipocytes can be formed from fibroblastic preadipocytes without altering their secretory profile and maintaining vascularization of the AT microenvironment (37, 38), which is associated with better metabolic health (39). A transcriptional cascade regulates this cell line differentiation carried out by peroxisome proliferator-activated receptor gamma (PPAR γ) and CCAAT enhancer-binding proteins (C/EBP), in conjunction with pro-adipogenic factors such as bone morphogenetic proteins (BMPs) (40, 41). However, hypertrophy and subsequent adipocyte dysfunction disrupt these signaling processes and preserve the pro-inflammatory phenotype characteristic of obese individuals (**Figure 1**) (42, 43).

The pre-existing adipocytes gain volume *via* increased fat accumulation, experiencing heightened mechanical stress by contact with adjacent cells and other extracellular matrix components (ECM) (44). Over time, AT expansion results in reduced regional blood flow, altered oxygen diffusion and finally tissue hypoxia, all of that related to both oxidative stress activation (OS) (45) and increased transcriptional activity of hypoxia-inducible factor 1α (HIF1α), nuclear factor kappa B (NF-kB), and cAMP response element-binding protein (CREB) genes, whose transcripts, in turn, drives to adipokines, chemokines, metalloproteases and growth factors gene expression, all of these related to a pro-inflammatory peptidic secretome (46). Concurrently, these hypoxia-induced factors downregulate anti-inflammatory and metabolism-regulatory adipokines such as adiponectin, which occurs alongside reduced transcription of antioxidant and thermoregulation-related genes, particularly catalase encoders UCP2, PPARγ and peroxisome proliferator-activated receptor-gamma coactivator 1α (PGC-1α) (47–49). Consequently, transcriptomic and proteomic changes in AT lead to a low-grade inflammatory environment characterized by functionally-altered fibroblasts,

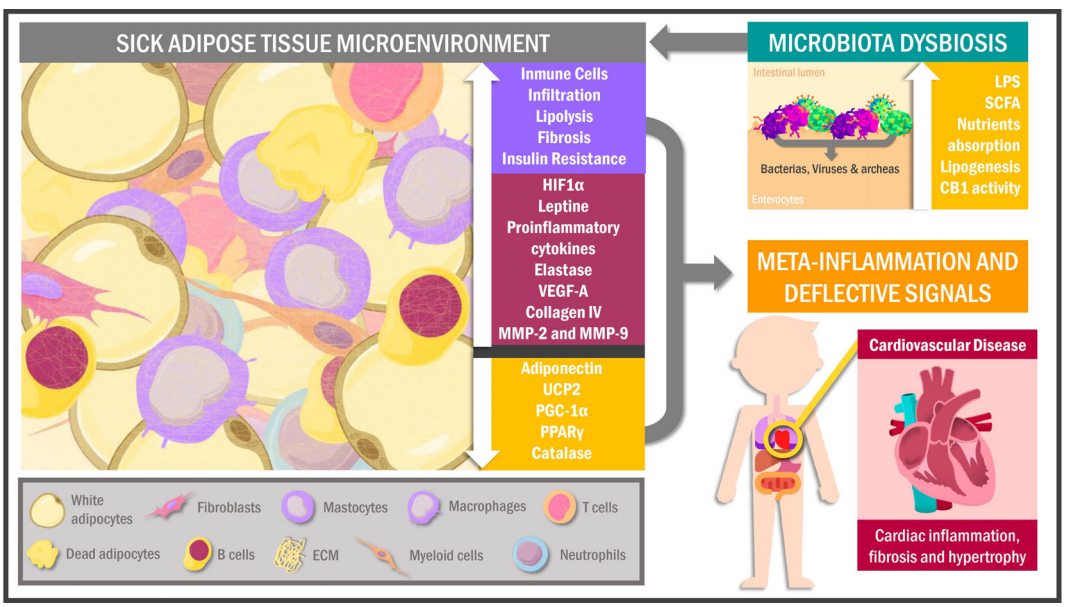

FIGURE 1 | Sick adipose tissue microenvironment and its interactions. Hypertrophic adipocytes and immune cells infiltration characterize the adipose tissue of obese individuals in response to a hypoxic environment as a signal for cell death and inflammation. This phenomenon leads to proteomic dysregulation and deflective peripheral signals promoting metabolic alterations in other tissues like muscle cells, particularly the myocardiocytes. In addition, obesogenic habits in overweighed people cause changes in the intestinal microbiota triggering adipose tissue chronic inflammation and cellular senescence. UCP2, uncoupling protein 2; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1α; PPARγ, peroxisome proliferator-activated receptor gamma; VEGF-A, vascular endothelial growth factor A; MMP, metalloproteinases; HIF1α, hypoxia-inducible factor 1α; LPS, lipopolysaccharides; SCFA, short-chain fatty acids; CB1, cannabinoid receptor 1; ECM, extracellular matrix.

endothelial cells and immune cells niche (50, 51). Regarding the latter, macrophages have been identified as the predominant cells of this system in AT, showing a pro-inflammatory M1 phenotype in obese individuals compared to the anti-inflammatory M2 in lean individuals (52). In this scenario, the hypoxic inflammatory state of AT promotes the release of interferon-γ (IF-γ) by T helper 1 (Th1) lymphocytes, inducing M1 macrophage recruitment and polarization, which causes increased release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein 1 (MCP-1), interleukin (IL) -6, IL-12, IL-1β and IL-23 (53–56).

Obese patients exhibit clusters of lipid-binding macrophages from dead adipocytes, a phenomenon well-correlated with AT inflammation and insulin resistance (57, 58). In addition, obesity promotes CD8 + and CD4 + T lymphocytes infiltration together with effector B-cells, heightening pro-inflammatory factors release and consequently AT dysfunction with defective extracellular signaling (59, 60). Similarly, myeloid cells, mast cells (50, 51) and neutrophils are also present in SickAT, showing that they contribute to tissue damage through elastase secretion and thus promote macrophage recruitment (61, 62).

Among the essential SickAT characteristics in obese patients are altered angiogenesis and endothelial dysfunction (ED). Although SickAT upregulates vascular endothelial growth factor A (VEGF-A) and HIF1α expression (both linked to angiogenesis), production is insufficient to generate neovascularization and counteract hypoxia, inflammation and necrosis characteristic of obese patients (63–66). Furthermore, SickAT leads to reactivity in the endothelium of the surrounding vessels, inducing the synthesis of intracellular adhesion molecule (ICAM-1), P-selectin and E-selectin, which in turn promotes macrophage infiltration worsening the pro-inflammatory milieu (67). Additionally, adipocyte-endothelial crosstalk can contribute to vasomotor alterations, deteriorating the oxygen bioavailability in EAT, PVAT and PAT (68, 69).

Likewise, HIF1α upregulation, immune cell infiltration and hyperactivity are associated with AT fibrosis. Remarkably, the increased synthesis of ECM components, mainly type-VI collagen and its cleavage products such as endotrophin, have been associated with metabolic dysfunction in obese mice *via* mechanical stress caused by limits on AT expansion (44, 70–72). Interestingly, HIF1α expression is correlated with metalloproteinases (MMP) -2 and MMP-9 in EAT, which are considered necessary for expansion and secretome alterations (69).

On a different plane, adipocyte metabolic activity is substantially modified in a hypoxic state. In fact, some glycolytic enzyme genes such as hexokinase 2 (HK2), phosphofructokinase (PFKP) and GLUT1 exhibit an increased expression in adipocyte cell cultures under hypoxic conditions (73, 74). Furthermore, although GLUT4 is the main isoform found in adipocytes, GLUT1 is the most efficient glucose transporter at low-oxygen levels (75). As expected in hypoxic states, the above changes suggest adipocytes have increased glucose uptake and metabolism (76), as confirmed by their increased lactate secretion (77).

In summary, lipid metabolism proteomics tends towards lipolytic extreme under hypoxic conditions (78). The SickAT microenvironment is characterized by multiple agents influencing insulin signaling, like IL-6, TNF-α, resistin, and IL-1β (79). Under normal conditions, insulin inhibits lipolysis through the mTORC1-Egr1-ATGL pathway, so inhibition of insulin´s second messengers cascade increases lipolytic activity (80). Furthermore, fatty acid uptake by adipocytes is blunted under hypoxic conditions (74), leading to plasma free fatty acids increase worsening insulin signaling (81) and contributing to the pro-inflammatory state (82). It should be highlighted that intrathoracic and visceral AT, BAT, BeAT and SCAT depots are affected in obesity (83), the thermogenic properties of BAT can be disturbed by mild inflammatory cells infiltration in severely obese individuals (84), leading to diminished glucose and FFA oxidative metabolism, and therefore contribute to IR and dyslipidemia development (84–86). In contrast, BeAT occurs less frequently owing to the dysfunctional state of WAT in obese patients (87).

2.2 Microbiota Dysbiosis

The gastrointestinal tract contains a complex population of microorganisms, the gut microbiota (GM), which exerts a marked influence on human health and disease (88). Multiple factors contribute to establishing the intestinal microbiota during early childhood and as it evolves into adulthood, but it is not hard to imagine that one of the main factors that shape the gut microbiota structure throughout our lives is our diet. In addition, gut bacteria play a crucial role in maintaining and proper function of the immune system and intermediary metabolism. Abnormalities in the intestinal bacterial composition (dysbiosis) have been associated with many inflammatory, infectious, autoimmune and metabolic diseases.

GM is constituted by bacteria, *archaea*, viruses and fungi, interacting symbiotically with the host (88). However, hypercaloric diet (HCD) and obesogenic habits alter the microbiota-host relationship, affecting its composition and interaction with the organism (89). A growing body of evidence in this area has centered on comparing energy and body fat storage in germ-free mice with transplanted microbiota of wild mice or obese individuals. The findings were that although the mice maintained the same diet in both cases, there was a substantial increase in adiposity and IR development after microbial transplantation, which could be attributed to the role of the microbiota in calorie extraction and absorption (90–92). Although the possible mechanisms triggered by HCD and obesity involved in the GM-AT axis interaction have not been fully elucidated yet, specific hypotheses have been proposed to explain these findings.

Significant among these theories is the influence of microbial products on AT. In physiological situations, the intestinal wall has selective permeability due to the tight junction proteins between enterocytes; however, an HCD can decrease expression of these proteins and allow passage of lipopolysaccharides (LPS), bacterial products of gram-negative bacteria (93–95). Once in circulation, LPS spread throughout the body and act on type 4 toll receptors (TLRs) located in AT adipocytes and immune cells (96), activating pathways dependent on myeloid differentiation factor 88- (MyD88-) and TIR-domain-containing adapter-inducing interferon-β (TRIF). This process activates the nuclear translocation of NF-kB and the subsequent release of pro-inflammatory substances,

contributing to the typic low-grade inflammation seen in SickAT (97, 98). Furthermore, it has been reported that LPS/TLR4 pathway activation can decrease WAT browning (99) and adaptive thermogenesis (100). Another interesting observation is that GD can increase permeability by activating the intestinal endocannabinoid system, acting on its CB1 receptors associated with obesogenic habits (101).

Likewise, several commensal bacteria species of the GM ferment indigestible carbohydrates and fiber to obtain energy by forming short-chain fatty acids (SCFA) (102–104), mainly acetate, butyrate and propionate. These metabolites have key roles in energy metabolism (105) and immunomodulation (106), by acting on the family of free fatty acid receptors (FFAR), especially FFAR2 (GPR43) and FFAR3 (GPR41), located in gastrointestinal, nervous, and AT tissue (107). Therefore, GD present in obese individuals may lead to changes in SCFA levels and, by extension, SickAT-related metabolic alterations. Higher SCFA production has been reported to promote lipogenesis by activating carbohydrate responsive element-binding protein (ChREBP) and sterol regulatory element-binding transcription factor 1 (SREBP1), favoring weight gain in animals models (108, 109). Similarly, studies have shown that SCFAs can inhibit fasting-induced adipocyte factor (FIAF), which can suppress enzyme lipoprotein lipase (LPL) activity and thus increase triacylglycerol (TAG) storage and accumulation in AT (90, 91).

Additionally, SCFAs stimulates peptide YY (PYY) and glucagon-like peptide 1 (GLP-1) secretion, which in turn slow down the intestinal transit time and thus increase nutrient absorption (109, 110), influencing appetite control (111). Other GT-AT axis-related mechanisms such as the TMA/FMO3/TMAO signaling pathway (112), nucleotide-binding oligomerization domain-containing 1 (NOD1) and NOD2 (113) proteins, and modulation of the miRNA-181 family (114) have also been explored in the context of obesity and its possible implications in the switch to SickAT. However, given the lack of a proven causal link between microorganisms, their products and specific mechanisms in humans, together with the heterogeneity of GT and the fact that *Bacteroidetes* and *Firmicutes* are predominant in both obese and healthy individuals (115), further research is warranted in this area.

3 INTERCELLULAR SIGNALING BETWEEN ADIPOCYTES AND MYOCARDIAL CELLS

3.1 Adipokines

3.1.1 Leptin

Leptin is a peptidic hormone secreted by AT, so peripheral leptin levels tend to remain directly proportional to AT volume (116). Consistent with this finding, obese patients show elevated leptin levels, but signaling defects mean that appetite suppression is reduced or nullified (117). Thus, obesity-related hyperleptinemia has been suggested as an important factor in CVD genesis (118). From a molecular perspective, leptin plays a role in atherosclerosis initiation by the hyper-production of reactive oxygen species (ROS) in endothelial cells (119). The explanation of this phenomenon relies on increased fatty acid oxidation *via* protein kinase A stimulation, which increases MCP-1 production, facilitating macrophage infiltration into the sub-endothelial (120). Furthermore, *in vitro* studies have shown that leptin increases cholesterol uptake in macrophages by ACAT1 modulation (121). These results match with clinical findings obtained in other studies; indeed, leptin levels are correlated with markers of atherosclerosis such as the intima-media thickness of the carotid artery (122) and likewise with the severity of coronary artery disease (CAD) (123).

It has also been hypothesized that leptin can induce cardiomyocyte hypertrophy (124). This effect seems mediated by multiple mechanisms, such as increased endothelin 1 (ET-1) and ROS production in cardiomyocytes in response to leptin levels (125). Another theory is that leptin activates the mTOR (126) and PPAR-α signaling pathways (127). Consistent with the above, clinical studies have shown a positive correlation between serum leptin levels and left ventricular thickness in obese or insulin-resistant patients (128). In contrast, another study conducted in a murine model proposes that leptin exhibits antihypertrophic properties. Based on these findings, mice with left ventricular hypertrophy reverted to normal ventricular function when normal leptin levels were restored (129).

Nonetheless, rather than a direct consequence of restored leptin levels, these findings may stem from reversing metabolic alterations inherent to leptin deficiency, so these results should be interpreted cautiously. On the other hand, the antihypertrophic properties associated with leptin levels have been reported in some studies (130–132). In conclusion, it remains uncertain whether cardiac hypertrophy is due to leptin pro-hypertrophic action or is instead an effect of resistance to leptin antihypertrophic action on cardiac remodeling.

3.1.2 Interleukin 6

As AT produces around a third of circulating IL-6, it can be considered an adipokine (133); however, its role in cardiomyocyte function is somewhat controversial. In acute phases, IL-6 signaling has been attributed a cardioprotective effect by inducing anti-apoptotic pathways and conferring protection against OS (134). However, IL-6 also decreases myocardial contractility and eventually increases nitric oxide (NO) production may be through inducible nitric oxide synthase (iNOS) activation (135, 136). Likewise, a study in animals reported no significant effects of treatment with IL-6 on left ventricular remodeling (137), while another study found that IL-6 signaling blockade suppresses myocardial inflammation and ventricular remodeling (137). Since human and murine IL-6 show only 41% similarity, animal studies should be approached with caution. Regarding human studies, elevated IL-6 levels have been correlated with ventricular dysfunction (138), heart failure, arrhythmias and worse clinical outcomes (139), indicating a need for further study to clarify the role of IL-6 in CVD.

3.1.3 Adiponectin

Under SickAT conditions, adiponectin secretion is considerably reduced, impacting negatively on cardiovascular function (140). On the other hand, normal adiponectin levels have been shown to

improve cardiomyocyte dysfunction in animal models, probably due to mechanisms related to IRS-1 and the c-Jun pathway (141). Furthermore, adiponectin is necessary to activate PPARγ signaling, which confers protection against myocardial hypertrophy and cardiac remodeling (142). Likewise, adiponectin inhibits iNOS and NADPH oxidase expression, decreasing OS under ischemic conditions (143). On a different level, adiponectin stimulates COX-2 expression and prostaglandin E2 synthesis, conferring cardioprotective and anti-inflammatory properties (144).

Clinically, hypoadiponectinemia is independently associated with ED (145), while normal adiponectin levels are associated with a lower risk of ischemic events in men (146). Conversely, low adiponectin levels positively correlate with left ventricular hypertrophy, regardless of age or other metabolic factors (147). However, a systematic review found no significant relationship between adiponectin levels and cardiovascular mortality, and a 10% increased risk of death from any cause was reported (148). This finding requires considering concurrent situations such as kidney failure and age-related adiponectin resistance, leading to bias when analyzing different populations (149, 150). Nonetheless, the prevailing view in the literature is that adiponectin confers cardioprotection at normal concentrations, while hypoadiponectinemia is related to an increased risk of developing ED as well as myocardial dysfunction.

3.2 BCAAs

Branched-chain amino acids (BCAAs), valine, leucine and isoleucine, are essential amino acids playing a critical energetic role in different tissues, including myocardial cells and adipocytes (151). For example, in physiological circumstances, adipocytes oxidize BCAAs as an important energy substrate; however, different stimuli or organic conditions such as insulin resistance, obesity or cardiovascular disease cause adipose cells reprogramming, reducing BCAA metabolism in the heart, AT and liver (152–154).

The mechanisms underlying these changes have not been fully elucidated yet; however, epigenetic changes such as PP2Cm, KLF15, or GRK2 gene expression during heart disease could modify the cells' metabolic profile. Subsequently, alterations in BCAA catabolism and use caused by these metabolic changes could lead to rising arterial amino acid levels (27, 153). Likewise, AT inflammation has been linked to tricarboxylic acid cycle modifications, resulting in reduced BCAA catabolism and use, which provides an alternative explanation for the accumulation of amino acids in plasma (155, 156) (**Figure 2**). These variations in local and organic BCAA concentrations lead to chronic mTOR receptor expression in myocardial cells, and thus, autophagy suppression pathways induction, alterations in insulin sensitivity and tissue transport, as well as protein synthesis pathway activation, promoting the inhibition of autophagy protective functions, by modifying the bioenergetic heart homeostasis and cardiac hypertrophy stimulation, respectively (157).

Given these findings, it is not surprising that a correlation between heart failure and elevated BCAA levels has been found in numerous studies (152, 158). For example, a clinical trial conducted by Peterson et al. evaluated total amino acid concentrations in patients with heart failure, finding them to be abnormally high (159). Similarly, results reported by Kato et al. indicated elevated plasma amino acid levels as a consequence of metabolic changes in sodium-sensitive hypertensive rodents (160). In contrast, a clinical trial conducted by Aquilani et al. reports a decreased BCAA levels in patients with chronic heart failure compared to healthy individuals. Although these results could seem contradictory, factors such as the site of amino acid quantification and the variability in BCAA levels due to both duration and severity of pre-existing disease could explain the differences between findings (161). In this regard, AT and cardiac tissue exert a reciprocal influence on each other in various pathological scenarios *via* modifications in BCAA catabolism and consumption (154–156, 162).

3.3 Cardiokines

The heart is conventionally viewed as a contractile organ acting as a muscular pump to provide nutrients to the body (163). Beyond these functions, however, it can exert regulatory actions on other organs, such as the kidney, liver or AT (164). These modulatory activities are carried out mainly through molecules synthesized and secreted by the heart, known as cardiokines (165–168).

To date, it has identified up to 16 cardiokines, which are thought to exert homeostatic functions related to growth, cell death, fibrosis, hypertrophy and cardiac remodeling. In addition, although these molecules have predominantly paracrine and autocrine functions, certain cardiokines show endocrine mechanisms of action, allowing them to act on distant tissues (169). Such is the case of the firsts cardiokines identified, known as atrial (ANP) and brain (BNP) natriuretic peptides (NPs) (164, 170). Besides their participation as blood pressure regulators, both peptides play a critical role in modulating AT energy metabolism (171).

In this way, different stimuli such as ischemia, reperfusion, OS, hemodynamic stress, and cardiac hypertrophy can trigger NP synthesis and release by different cardiac cells into the circulation and ultimately reaching the AT (172). In this tissue, NPs bind to the NPR-A receptors, activating the guanylyl cyclase and cGMP formation. This process, in turn, activates the PKG, an enzyme responsible for phosphorylating key factors such as UCP-1, PPARGC1A, CYCS, PRD1-BF 1 and RIZ1, inducing white adipocyte browning, increasing lipogenesis, mitochondrial biogenesis and lipid oxidation (171, 173).

Collectively, these phenomena have a double effect. Fatty acids are released into the bloodstream as energy substrates to compensate for the low heart contractility observed during the abovementioned pathological scenarios (174), while increased mitochondrial production, thermogenesis, and fatty acid oxidation promote weight loss (175, 176). These reports were verified by other studies showing abnormally elevated NP concentrations during CVD and decreased levels of these peptides in obese individuals (164, 177). For example, in one study carried out by Kovacova et al. (29), the NPRR expression was significantly lower in obese than normal-weight individuals. These findings replicated those obtained in studies carried out in humans and murine, wherein plasma and cardiac levels of both BNPs and ANPs were significantly lower in obese than normal-weight subjects (175, 178).

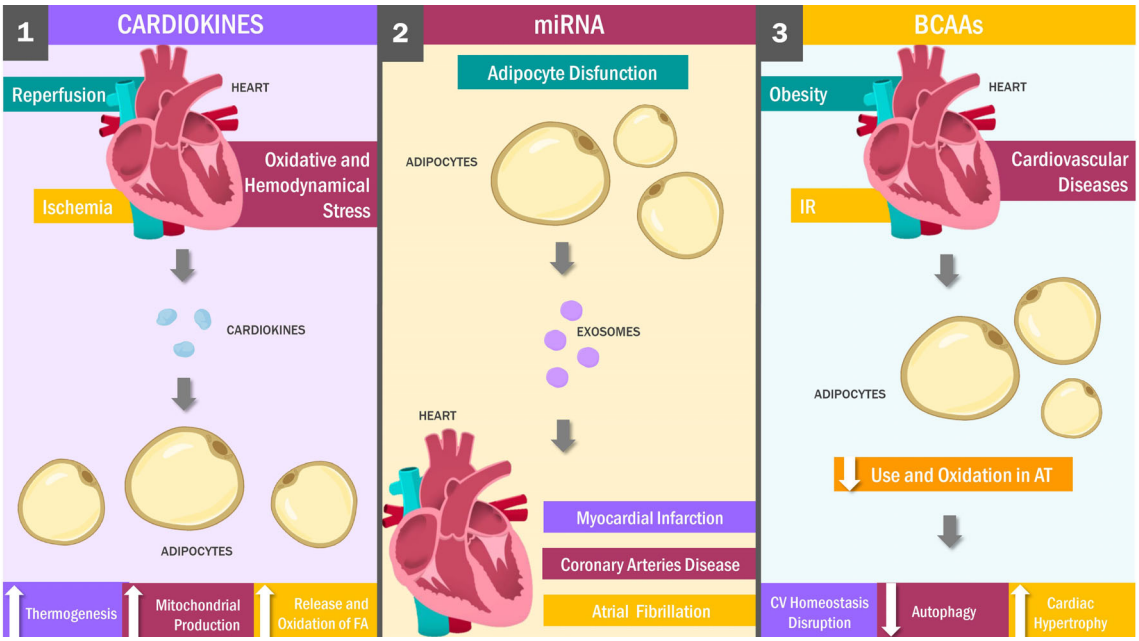

FIGURE 2 | Heart and Adipose Tissue Crosstalk: Key Messengers. Cardiokines: stimuli such as cardiac ischemia, reperfusion, oxidative and hemodynamic stress stimulate the production of cardiokines, which signaling in an endocrine and paracrine mechanism to the adipose tissue promoting weight loss by increasing thermogenesis and both the release and oxidation of fatty acids. Adipose tissue dysfunction is a stimulus for miRNAs release, which travel through the bloodstream to the myocardial tissue inside exosomes, exerting cardioprotective against myocardial infarction, coronary artery disease and atrial fibrillation. On the other hand, obesity, IR and cardiovascular diseases decrease BCAAs oxidation in adipose tissue, which decreases autophagy with heart hypertrophy and, finally, the alteration of the bioenergetic homeostasis of the heart. FA, Fatty Acids; AT, Adipose Tissue; CV, Cardiovascular; IR, Insulin resistance.

3.4 miRNAs and EVs

miRNAs are small, non-coding RNA molecules functioning as regulatory agents in numerous physiological and pathological processes by participating in post-transcriptional mRNA and translation into protein processes (179, 180). These molecules are synthesized in response to a wide range of stimuli by different tissues (181), among which AT and EAT are responsible for the production and release of multiple miRNA varieties (14, 182).

Most miRNAs on EAT operate through autocrine fashion and have been implicated in various AT processes such as adipocyte differentiation, fatty acid metabolism, cholesterol homeostasis, adipogenesis, browning and inflammation (183–185). Other miRNAs are released into the circulation *via* exosomes, from where they travel to and penetrate the heart or other distant organs (186). Although it has been established that EAT releases different miRNAs towards the heart in response to tissue dysfunction or certain specific stimuli (13), but the functions and underlying mechanisms of action have not been fully characterized. Nonetheless, recent studies have identified new miRNAs and their potential role in the pathogenesis and development of heart diseases (12, 187, 188).

In this vein, miRNAs have been implicated in atrial fibrillation (AF), as demonstrated in the study carried out by Liu et al. (189), wherein miR-320d were transported *in vitro* by exosomes to FA cardiomyocytes, revealing enhanced cell viability and decreased post-transfection cardiomyocytes apoptosis, reversing several FA characteristic effects by inhibiting factor STAT3. Likewise, a possible cardioprotective role has been suggested to miR-146a due to an inhibitory effect on early growth response factor-1 (EGRF1) in suppressing typical post-MI phenomena such as apoptosis, inflammatory responses and cardiac fibrosis (190). Similar results were obtained by Luo et al., in which miR-126 overexpression in hypoxic H9c2 cells led to reduced local inflammation, pro-fibrotic protein expression, and microvasculature and cell migration, thus mitigating the effects of cardiac injury in the infarcted area (191).

Numerous miRNAs play a positive role in some cardiac pathologies beyond acute myocardial infarction (AMI) and AF, including CAD. For example, it has been shown that during CAD progression, miRNA-3614 expression is downregulated in EAT, which produces an inhibitory effect on factors such as TRAF6, which regulates immune cell recruitment and activation as apoptosis and cardiac remodeling during myocardial ischemia (189, 192). In this context, a study by Zou et al. identified miR-410-5p and its promoting effects on cardiac fibrosis in mice with regular diets by silencing Smad7; concurrently, miR-410-5p demonstrated anti-fibrotic effects in mice fed high-fat diets (193). These results suggest a dual role for miRNAs in cardiovascular pathologies; besides the cardioprotective role of some miRNAs, these molecules can exert harmful effects on cardiac tissue, promoting effects such as local inflammation, hypertrophy, remodeling and cardiac fibrosis in different CVDs (183, 194–198).

4 NON-PHARMACOLOGICAL APPROACH TO ADIPOCYTE-MYOCARDIOCYTE DEFECTIVE SIGNALING: IMPACT OF LIFESTYLE

Preclinical and clinical evidence suggests that positive lifestyle changes derived from increased PA and NI could improve the above-described pro-inflammatory metabolic status of obese patients, highlighting their utility as possible non-pharmacological therapeutic strategies to manage obesity and cardiovascular risk.

In this regard, studies suggest that PA reduces circulating levels of insulin, leptin, and pro-inflammatory cytokines and raise adiponectin and apelin concentrations (199–202). In addition, increasing PA has been linked to heightened endothelial NOS (eNOS) expression and iNOS expression reduction (199, 203). These findings suggest that PA as a strategy helps restore a healthy metabolic state at the preclinical level.

Additionally, clinical studies have reported an anti-inflammatory, cardioprotective and slimming effect of PA. For example, a study in obese men showed that exercise was more effective than diet in reducing body weight (BW), improving the systemic inflammatory profile and IR and circulating levels of adipokines (204). Likewise, a study conducted in obese patients with T2DM subjected to dietary restriction and aerobic exercise reported that after a 3-month intervention, adiponectin levels rose while BMI and TNF-α, IL-6 and leptin levels fell significantly (205).

Concerning the different intensities of PA, a clinical trial demonstrated that moderate exercise combined with calorie restriction aided in normalizing adiponectin, leptin and resistin levels in obese adolescents (206). Furthermore, a meta-analysis performed by Maillard et al. (207) reported that high-intensity interval training effectively reduced SCAT and VAT. Similarly, another meta-analysis found that both moderate and high-intensity PA have a similar effect on weight reduction and body composition; however, results were seen more quickly when performing high-intensity exercise (208). Therefore, besides its anti-inflammatory properties, exercise can reduce BW, indirectly counteracting SickAT defective signaling by modifying its composition.

Studies have also demonstrated that PA has a regulatory effect on circulating microRNAs in individuals with cardiometabolic abnormalities. In this context, a clinical trial showed that circulating levels of miR-192 and miR-193b (associated with a prediabetic state) were modified after 16-week exercise intervention (209). Along similar lines, a combined aerobic and resistance exercise program in obese patients for three months was associated with significantly decreased levels of the inflammatory miRNA miR-146a-5p (210).

Aside from the weight loss achieved with exercise, dietary interventions have also been shown to positively impact AT and CVS crosstalk. In this regard, it has been proven that caloric restriction in the rat diet causes significantly reduced expression of iNOS, TNF-α and IL-1β in PVAT (211). Furthermore, another study conducted in rats showed that calorie control-induced weight loss was associated with improved endothelial NOS function, reduced TNF-α levels and normalized plasma adipokines y hormones levels such as leptin and insulin (212). Therefore, diet is a rationale tool to improve the cardiovascular functionality of the PVAT.

In another study, Kim et al. showed that intermittent fasting (IF) with an isocaloric diet increased VEGF expression in WAT, favoring macrophage polarization towards the M2 phenotype, which is linked to increased thermogenesis and AT browning (213). In this regard, a clinical trial in obese patients reported that IF combined with caloric restriction and liquid meals promotes significant BW loss and improves risk indicators for CAD (214). Furthermore, other studies conducted by the same research group (215) and Trepanowski et al. (216) were able to show that in addition to reducing BW, the abovementioned diet decreases levels of leptin IL-6, TNF-α and insulin-like growth factor-1 (IGF-1). These results point to IF and low-calorie diets as a possible strategy to manage AT visceral adiposity and secretory profile, owing to their cardioprotective effect.

Regarding the role of the nutritional maneuvers approach on circulating microRNAs, Hsieh et al. (217) showed through a preclinical study that a low-fat diet could reverse obesity-associated inflammatory miRNA profiles *via* BW reduction. Consistent with this finding, evidence in humans suggests that BW loss achieved by very-low-calorie NI in obese women (218) or protein-rich diets in obese men (219) allow positive modulation of circulating levels of different miRNAs such as miR-34a, miR-208, miR-193a, miR-223, miR-320, miR-433, miR-568 and miR-181a.

Likewise, preclinical and clinical studies have shown the prebiotic and probiotic effects in reducing cardiovascular risk by leptin resistance (220) and leptin level reductions (221, 222). In addition, an adiponectin increase (223, 224) and lowering both apelin (225) and ANP levels (226) have been consistently reported, a fact attributed to HCD-induced GD correction and thus a reduced LPS-induced endotoxemia and SCFA levels. Likewise, 3-n PUFA supplementation has been associated with recovery of the adipokine and cardiokine profile, resulting in a healthier cardio-metabolic state. In this context, studies in animal models and humans have linked supplements administration with a significant reduction in leptin (227, 228), follistatin-like 1 (229) and BNP levels (230), and adiponectin increase (231). Finally, polyphenols such as lycopene, resveratrol and curcumin have also been linked to improved inflammatory and adipokine profile, body composition and cardiac fibrosis/hypertrophy in study subjects (232–235).

These data suggest that PA and different NI, either alone or in combination, are associated with the upregulation of adipokines, cardiokines, miRNAs and other components associated with crosstalk between AT and CVS. Therefore, these strategies are beneficial in reducing cardiovascular risk in obese patients due to their mechanisms capable of counteracting the characteristic pro-inflammatory state of SickAT.

5 CONCLUSIONS

Adipose tissue is a multifunctional exhibiting well-characterized inter-organ paracrine and endocrine networking, including myocardial tissue communication. Obesity is characterized

by metabolic changes in SickAT caused by a hypoxic microenvironment due to adipocyte hypertrophy driving to immune cell infiltration and a systemic pro-inflammatory state affecting target cells such as cardiomyocytes. Excessive adipokines, microRNA, BCAAs characterize SickAT defective signaling, and other pro-inflammatory substances release altering myocardial cells function and, consequently, CVD development. Likewise, heart cells can also alter AT signals, thereby causing a vicious cycle that fuels meta-inflammation. Under this premise, lifestyle changes such as PA, low-calorie diets, IF, and food supplementation are fundamental non-pharmacological therapeutic tools to combat obesity and CVD due to their identified regulatory mechanisms in AT and CVS signaling.

AUTHOR CONTRIBUTIONS

Conceptualization: PD, MN, CC, and VB. Investigation: LD'M, MD, RC, MN, and MC. Writing – original draft: PD, MD, RC, MC, MB, JG, and ER. Writing – review and editing: VB, CC, JR, MB, and LD'M, JG. Funding acquisition: VB and JG. All authors contributed to the article and approved the submitted version.

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Sestrin2 as a Potential Target for Regulating Metabolic-Related Diseases

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Sestrin2 is a highly conserved protein that can be induced under a variety of stress conditions, including DNA damage, oxidative stress, endoplasmic reticulum (ER) stress, and metabolic stress. Numerous studies have shown that the AMP-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) signaling pathway has a crucial role in the regulation of metabolism. Sestrin2 regulates metabolism *via* a number of pathways, including activation of AMPK, inhibition of the mTOR complex 1 (mTORC1), activation of mTOR complex 2 (mTORC2), inhibition of ER stress, and promotion of autophagy. Therefore, modulation of Sestrin2 activity may provide a potential therapeutic target for the prevention of metabolic diseases such as insulin resistance, diabetes, obesity, non-alcoholic fatty liver disease, and myocardial ischemia/reperfusion injury. In this review, we examined the regulatory relationship between Sestrin2 and the AMPK/mTOR signaling pathway and the effects of Sestrin2 on energy metabolism.

Keywords: Sestrin2, AMPK, mTOR, metabolism, metabolic diseases

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INTRODUCTION

Sestrins, a family of evolutionarily highly conserved stress-induced proteins, are upregulated under oxidative stress, genotoxic stress, hypoxia, and other stress conditions (1). As stress-induced metabolic modulators, Sestrins help cells adapt to diverse stress stimuli by activating catabolic reactions, stopping anabolic activities, and initiating cell repair mechanisms, to maintain cell homeostasis (2). In mammals, there are three members of the sestrin family, Sestrins1–3, which are encoded by three independent genes, while only one Sestrin ortholog has been identified in invertebrates (3–5). Sestrin1, also referred to as p53-activated gene 26 (*PA26*), was first identified by Velasco-Miguel et al. and is a growth arrest and DNA damage-inducing gene (5). In 2002, Sestrin2, also known as hypoxia-inducible gene 95 (Hi95), was reported by Budanov et al., is highly homologous to Sestrin1, and can be induced by prolonged hypoxia and DNA damage (6, 7). Sestrin3 is directly activated by forkhead box O (FOXO) transcriptional factors (8). These three Sestrin proteins have some shared mechanisms of action, including, but not limited to, inhibiting the production of reactive oxygen species (ROS), activating AMPK, and inhibiting mTORC1 (4, 9). However, there is growing evidence that the three Sestrins behave differently and promote different biological effects *via* AMPK/mTOR signaling because they are distributed differently in different organs (10, 11). To our knowledge, Sestrin1 has an antioxidant function that can activate the AMPK signal pathway while inhibiting the mechanistic target of the mTORC1 signal pathway (12).

Furthermore, Sestrin1 can be activated in a p53-dependent manner under oxidative stress in skeletal muscle, kidney, brain, and lung (7). Recent studies suggest that Sestrin1 inhibits oxidized low-density lipoprotein-induced activation of NOD-like receptor protein 3 (NLRP3) inflammasome in macrophages in a murine atherosclerosis model (12). What is even more interesting is that in multiple mouse models, Sestrin1 influences plasma cholesterol and regulates cholesterol biosynthesis (13). Among these members, Sestrin2 is the most intensively studied since its discovery in 2002. As a p53 target gene, Sestrin2 (*SESN2*) can exert cytoprotective functions in the lung, heart, liver, adipose, and kidney through activation of AMPK and inhibition of mTORC1 (6, 11, 14, 15). Furthermore, Sestrin2 is able to suppress nitric oxide release and the production of classical pro-inflammatory cytokines in cardiomyocytes (16). Sestrin3 can inhibit mTORC1 and maintain the activity of protein kinase B (AKT) *via* activating the AMPK/TSC1/2 signaling pathway (8). Sestrin3 is largely expressed in skeletal muscle, intestine, adipose, colon, and brain (17).

Increasing evidence suggests that that Sestrin2 has two main biological functions. Through its own oxidoreductase activity or activation of antioxidant damage related pathways, Sestrin2 can reduce the damage of oxidative stress to protect cells and tissues and maintain redox homeostasis (18, 19). In addition to its redox activity, Sestrin2 can also inhibit the mammalian target of mTORC1 through AMPK-dependent or -independent pathways (20). These two activities of human Sestrin2 (hSestrin2) are supported through its two separate domains, which were determined from X-ray crystallographic studies. A recent study of the X-ray crystal structure of hSestrin2 showed that it consists of well-conserved Sesn-A, Sesn-B, and Sesn-C domains (11). Sesn-A and Sesn-C are structurally similar but functionally distinct from each other (21). Sestrin2 controls ROS and mTOR signaling through two separate functional domains (22). While Sesn-A reduces alkyl hydroperoxide radicals through its helix–turn–helix oxidoreductase motif, Sesn-C modifies this motif to accommodate physical interactions with GAP activity towards Rags 2 (GATOR2) and subsequent inhibition of mTORC1 (21, 23). Sestrin2 has a significant role in the inhibition of ER stress and the activation of autophagy and is considered to improve obesity-induced and age-related pathologies by inhibiting mTORC1 (15, 24). Therefore, Sestrin2 may represent a novel class of potential targets for the therapeutic intervention of metabolic diseases. In this review, we discuss the regulatory relationship between Sestrin2 and AMPK/mTOR signaling and the effects of Sestrin2 on energy metabolism.

REGULATION OF SESTRIN2 EXPRESSION IN RESPONSE TO DIVERSE STRESS CONDITIONS

Human beings exist in a constantly changing environment and face frequent challenges that threaten our survival and health. In response to stress, the body undergoes very subtle changes at the cellular and molecular level. Understanding how Sestrin2 is regulated under different stress conditions is very helpful for us in studying Sestrin2. Therefore, it is of great significance to study the regulatory mechanism of Sestrin2 expression under different types of stress conditions.

Sestrin2 and Oxidative Stress

Reactive oxygen species and reactive nitrogen species (RNS) are generated continuously in the body through oxidative metabolism, biological functions of mitochondria, and immunologic functions (25). Physiological ROS are crucially important for intracellular and extracellular signal transduction (26). However, it is well-known that overloaded ROS and RNS can bind with and destroy most cellular biomolecules (lipids, enzymes, sugars, proteins, nucleic acids, and other small molecules) under oxidative stress (27–29). Oxidative stress is considered to be an imbalance in redox properties in certain cellular environments (30), and plays a crucial role in the development of numerous human diseases, including diabetes, obesity, and myocardial injury (31–33). Resistance to oxidative stress injury is one of the important functions of Sestrin2. In response to oxidative stress, the expression of Sesrin2 is regulated at the mRNA and protein level by various transcription factors, including nuclear factor kappa-B (NF-κB), activator protein-1 (AP-1), CCAAT-enhancer-binding protein beta (C/EBPβ), forkhead box O3 (FOXO3), and p53 (19, 24, 34–36). Sestrin2 has been suggested to maintain the balance of oxidative metabolism through two main biological functions. First, as an antioxidant enzyme, Sestrin2 is capable of directly reducing the accumulation of ROS (37). However, the intrinsic catalytic antioxidant activity of Sestrin2 remains elusive and limited. Second, recent studies have demonstrated that Sestrin2 inhibits ROS production and defends cells against oxidative stress, which is likely to be mainly attributed to its regulation of several signaling pathways related to oxidative stress: the Kelch-like Ech-associated protein 1 (KEAP1)/NF-E2 related factor-2 (NRF2) antioxidant signaling pathway (2) (**Figure 1**) and the AMPK and mTORC1 pathways (which will be described in detail later) (38). NRF2 is a transcription factor that can bind to antioxidant-responsive elements (AREs) to promote the expression of many antioxidant molecules to protect cells from oxidative insults (36). NRF2 is constitutively expressed in the cytoplasm under physiological conditions (39). Under normal conditions, KEAP1 binds to NRF2, preventing NRF2 translocation to the nucleus, promoting its ubiquitination and proteasome degradation, and maintaining free NRF2 in the cytoplasm at a low level (19). Under oxidative stress, NRF2 dissociates from KEAP1 and translocates to the nucleus. NRF2 binding to ARE activates the transcription of target genes *PRX*, *SRX*, superoxide dismutase (*SOD*), catalase (*CAT*), heme oxygenase 1 (*HO1*), and glutathione peroxidase 1 (*GPX1*) (40, 41). In cellular studies, it was found that Sestrin2 binds to unC-51-like kinase 1 (ULK1) and p62 to form functional complexes, and that Sestrin2 promotes the phosphorylation of p62, which further promotes KEAP1 degradation and NRF2 activation (42). In addition, in studies of liver damage caused by oxidative stress, Sestrin2 was shown to act as a scaffold protein to enhance the

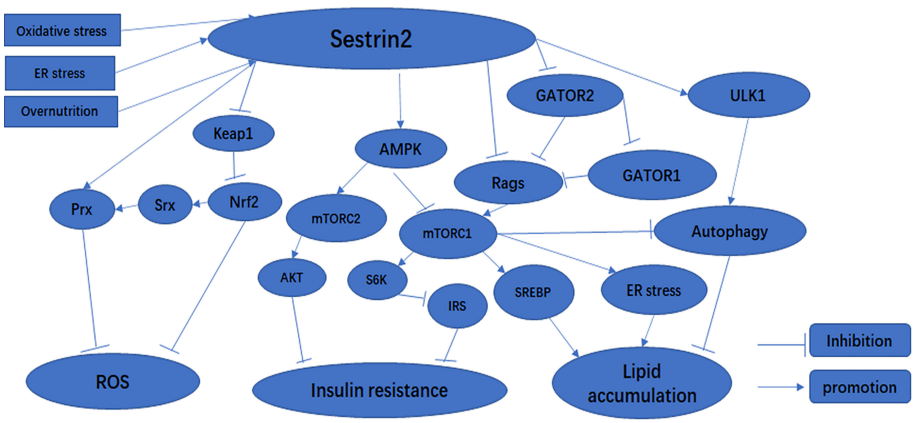

FIGURE 1 | The effect of Sestrin2 on metabolic-related signaling pathways.

weak binding of KEAP1 to p62, thereby promoting KEAP1 autophagy degradation and preventing oxidative liver injury (43). More interestingly, NRF2 regulates the expression of Sestrin2 by binding to the ARE promoter of *SESN2* under oxidative stress (37). A positive feedback loop is formed between Sestrin2 and NRF2 to promote the transcription and translation of antioxidant-related genes downstream of NRF2 and to protect cells from oxidative damage (36). Therefore, during oxidative stress, Sestrin2 is crucial to maintaining cellular homeostasis.

Sestrin2 and Endoplasmic Reticulum Stress

ER stress occurs when unfolded or misfolded proteins accumulate in the endoplasmic network lumen due to adverse physiological conditions (44). During ER stress, cells can improve their protein folding ability, inhibit protein production and accumulation, induce ER stress-related gene transcription, and strengthen the self-repair ability of ER to restore protein-folding homeostasis and regulate ER homeostasis through a series of transduction pathways, including protein kinase R-like endoplasmic reticulum kinase-eukaryotic translation-initiation factor 2α (PERK-eIF2α), inositol-requiring enzyme 1a-X-box-binding protein 1 (IRE1-XBP1), and activating transcription factor 6-CREBH (ATF6-CREBH). These reactions are called the unfolded protein reaction (UPR) (45). If ER stress is too strong or lasts too long, these responses are not enough to restore ER homeostasis, and apoptosis is eventually induced (46). A growing body of research has demonstrated that expression of Sestrin2 can be upregulated under ER stress conditions (15, 35, 47). For instance, Park et al. (15) found that upregulated Sestrin2 is associated with an ER stress-activated transcription factor, CCAAT enhancer-binding protein beta (c/EBPb). Once induced, Sestrin2 in turn stops protein synthesis by inhibiting mTORC1. Recently, a study by H. Jeong Kim et al. (48) revealed that induction of Sestrin2-regulated genes can be connected *via* activation of the PERK/eIF2α/ATF4 pathway. Consistent with these findings, Jegal et al. (35) demonstrated that under ER stress, expression of Sestrin2 can be enhanced *via* activating transcription factor 6 in hepatocytes, and Sestrin2 decreases the phosphorylation of JNK and p38 as well as PARP cleavage, and blocks the cytotoxic effect of excessive ER stress so as to play a hepatoprotective role both *in vitro* and *in vivo*. Furthermore, Ding et al. (49) elucidated that upregulation of Sestrin2 expression is dependent on ATF4 and NRF2 but not p53 under ER stress induced by glucose starvation. To summarize, Sestrin2 might serve as an important regulator that exerts cell and tissue protection functions under excessive ER stress. However, the exact mechanism by which ER stress induces Sestrin2 expression remains poorly understood and requires further exploration.

Sestrin2 and Obesity

Obesity is traditionally considered to be the excessive accumulation of fat in the body, which is a serious hazard to human health, and in clinical practice, obesity is usually assessed by the body mass index (BMI) (50). With the improvements in the general standard of living, the incidence of obesity has risen sharply (51). Obesity is a major contributor to the development of metabolic syndromes, including type 2 diabetes mellitus, hypertension, hyperlipidemia, and cardiovascular disease (52). Studies have shown that overnutrition and a sedentary lifestyle are the main causes of obesity (53). mTORC1 is a nutrient-sensitive protein kinase that has a fundamental role in maintaining metabolic homeostasis (54). Recent research has clarified that overnutrition can result in chronic mTORC1 activation (55). In response to persistent overnutrition, chronic mTORC1 activation can enhance protein and lipid biosynthesis and inhibit autophagic catabolism (56). Several studies confirmed that chronic mTORC1 activation mediated by stress responses such as overnutrition ultimately leads to overexpression of Sestrin2 (24, 57). Lee et al. (24) found that the expression of *Drosophila* Sestrin (dSesn) is upregulated upon chronic mTORC1 activation *via* the c-Jun N-terminal kinase (JNK) and FOXO signaling pathways. Loss of dSesn results in

triglyceride accumulation and mitochondrial dysfunction. Furthermore, another study by Lee et al. (57) demonstrated that Sestrin2 is the only Sestrin protein that is induced by overnutrition and obesity and attenuates chronic mTORC1 activation *via* the mTORC1/S6K axis in mouse liver. In agreement with these conclusions, Kimball et al. (58) revealed that Sestrin2 expression was upregulated in the livers of rats fed with a high-fat diet. Thus, in a nutshell, Sestrin2 exerts important metabolic homeostatic functions.

SESTRIN2 AND THE AMPK/MTOR SIGNALING PATHWAY

mTOR is an evolutionally conserved protein that is a critical regulator of cell proliferation, proliferation, metabolism, and autophagy (37). mTOR promotes anabolic processes such as ribosome biogenesis and synthesis of proteins, nucleotides, fatty acids, and lipids, and inhibits catabolic processes such as autophagy (54). It is composed of two structurally and functionally distinct complexes, mTORC1 and mTORC2, which are characterized by the presence of Raptor and Rictor, respectively (59). mTORC1 consists of mTOR Raptor, PRAS40, and mLST8, while mTORC2 is composed of mTOR, Rictor, Sin1, Protor, and mLST8 (54). mTORC1 promotes protein and lipid synthesis through the phosphorylation of its distinctive substrates, such as ribosomal protein S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein 1 (4EBP1) (2). In addition, mTORC1 may also regulate adipogenesis through the regulation of the sterol regulatory element-binding proteins (60). Furthermore, mTORC1 can phosphorylate and suppress autophagy-initiating protein kinases unc-51-like kinase 1 (ULK1) to inhibit cellular autophagic catabolism (61). mTORC2 regulates metabolism and cytoskeletal tissue in response to growth factors through the activation of AGC family kinases, including AKT, SGK1, and PKCα (62). Recent studies have shown that mTORC2 in particular is a crucial controller of lipid metabolism that regulates adipogenesis in the liver (60).

AMPK, an important nutrient-sensing protein kinase, has a critical role in increased catabolism and decreased anabolism (63). AMPK can inhibit the phosphorylation of the acetyl-CoA carboxylases ACC1 and ACC2, HMG-CoA reductase, and the glycogen synthases GYS1 and GYS2 to regulate the biosynthesis of glycogen and lipids (63). It can also inhibit mTORC1 activity through the phosphorylation of its regulatory subunit Raptor (64) or through the phosphorylation of tuberous sclerosis complex 2 and inhibition of mTORC1-activating guanosine triphosphatase (GTPase) Rheb (4). In addition, AMPK restrains the transcriptional activity of sterol regulatory element binding protein (SREBP) through direct phosphorylation to decrease the expression of lipogenic genes (65).

Once induced by stress, Sestrin2 affects a variety of signaling pathways, thus upregulating stress adaptation mechanisms (23). When induced in response to oxidative stress, Sestrin2 inhibits mTORC1 through the activation of AMPK (66). Consequently, Sestrin2-deficient cells and tissues exhibit lower AMPK and higher mTORC1 activity under both normal and stressed conditions (6, 24, 66). It has been reported that Sestrin2 acts as a scaffold protein, promoting the binding of LKB1 to AMPK and subsequent AMPK phosphorylation and activation, and controls mTORC1 signaling as an inhibitor of guanine nucleotide dissociation in Rag GTPases (6, 67, 68). Sestrin2 can also activate AMPK through direct interaction with the α subunit of the AMPK complex (66).Recent studies have shown that Sestrin2 can inhibit mTORC1 through AMPK-dependent or -independent pathways (15, 20, 57, 68) (**Figure 1**). Sestrin2 can also modulate amino acid-stimulated mTORC1 activation through direct interactions with Rag A/B GTPases or GATOR2 complexes (68, 80). Sestrin2 binds to GATOR2 and releases GATOR1 from GATOR2-mediated inhibition. Released GATOR1 subsequently binds to and inactivates RagB, ultimately resulting in mTORC1 suppression (81) (**Figure 1**). In addition, Sestrin2 plays a critical role in the activation of autophagy through multiple mechanisms including activation of AMPK, inhibition of mTORC1, and activation of ULK1 (82) (**Figure 1**). Therefore, the AMPK/mTORC1 signaling pathway is critical for Sestrin2 in controlling cell metabolism and survival under stress conditions (**Figure 1**).

ROLE OF SESTRIN2 IN DIABETES, NON-ALCOHOLIC FATTY LIVER DISEASE, AND MYOCARDIAL ISCHEMIA/REPERFUSION (I/R) INJURY

Mounting evidence has demonstrated that Sestrin2 is upregulated in response to diverse stress conditions, including oxidative stress, ER stress, and metabolic stress. Sestrin2 exerts a significant influence on the protection of human cells and tissues *via* related signal transduction pathways, and was shown to play a critical role against metabolic diseases, such as diabetes, obesity-related non-alcoholic fatty live, and myocardial I/R injury (**Table 1**).

Sestrin2 and Diabetes

Diabetes is the most common metabolic disease, and is a chronic disease characterized by persistent hyperglycemia (83). More than 90% of diabetics have type 2 diabetes, and insulin resistance is consistently found in patients with type 2 diabetes (84). Insulin resistance is an impaired biological response to insulin stimulation in target tissues, primarily liver, muscle, and adipose tissue (85). Insulin resistance impairs glucose processing, leading to a compensatory increase in beta cell insulin production and hyperinsulinemia. Sestrin2 is highly expressed in the liver (86). According to literature reports, two pathways of Sestrin2 affect cell signaling pathway transduction: one activates the AMPK pathway and the other downregulates the mTOR pathway (2, 87). AMPK is an enzyme activated in energy-deficient conditions (2). Sestrin2 is induced by oxidative stress through activation of the NRF2 and JNK/AP-1 signaling

TABLE 1 | Summary of the role of Sestrin2 in metabolic diseases.

Disease	Signaling pathway	Effect	Reference
Diabetes	AMPK/mTOR	Improves insulin resistance	(57, 69)
		Increases insulin-sensitivity	
Nonalcoholic fatty liver disease	AMPK/mTORC1	Reduces lipid synthesis	(24, 70)
		Attenuates ER stress	(15)
	Nrf2/ Keap1	Promotes autophagy	(2)
	Nrf2/HO-1	Prevents oxidative liver damage	(43)
	JNKs	Keeps redox balance	(71)
		Attenuates lipotoxicity	(72)
Myocardial ischemia/reperfusion (I/R) injury	AMPK/PGC-1α	Reduces the area of myocardial injury	(73)
		Attenuates the sensitivity of myocardium to ischemia	
	AMPK/LKB1	Protects mitochondrial biogenesis	(74)
		Inhibits myocardial cell apoptosis	(75)
	MAPK signaling pathway	Diminishes myocardial infarct size	(76)
	Antioxidant protein	Improves function of infarcted myocardium	(77)
	AMPK/mTOR	Refines myocardial substrate metabolism	(78)
		Modulates cardiac inflammation	(79)
		Restrains ROS production	
		Improves contractile function	
		Attenuates myocardial hypertrophy	
		Improves cardiac function	

axes (4, 43, 88). In bacteria, AhpD is a critical member of the antioxidant defense system and regenerates peroxide AhpC, a bacterial peroxidant protein (Prx), through catalytic reduction. In mammalian cells, Sestrins interact with overoxidized PRX and promote its regeneration. Here, Sestrins act similarly to AhpD in bacteria (77). Sestrins have no direct catalytic activity leading to the reduction of PRX, but may regenerate PRX by promoting the activity of other oxidoreductases, such as thioredoxin (SRX) (4). Sestrins can increase SRX expression by activating NRF2 (6, 43) (**Figure 1**). Increased glucose downregulates Sestrin2 expression, thereby increasing mTOR activity and inhibiting AMPK (87, 89). Moreover, when treated with high levels of glucose, such as metformin (an AMPK agonist and mitochondrial respiratory inhibitor), Sestrin2 was upregulated, mTOR activity was significantly increased, and AMPK activity was decreased (6, 87). S6K is an effector of the mTOR pathway (90). By activating S6K, mTORC1 promotes insulin resistance by inhibiting phosphorylation of insulin receptor substrates (IRS) (**Figure 1**) and attenuating the insulin-induced phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway (6). mTORC1/S6K activity leads to serine phosphorylation and protein degradation of IRS, forming a negative feedback loop in which insulin signaling attenuates subsequent insulin action (89). Lack of amino acids, especially leucine, leads to rapid dephosphorylation of the mTORC1 effectors S6K and 4EBP1, which depend on mTORC1 for amino acid resynthesis (91). Sestrin2 is required to maintain insulin sensitivity in the liver in high-fat diet (HFD)-induced dietary obesity and Lepob mutation-induced inherited obesity (57). AMPK and mTORC1 are important protein kinases with complete antagonistic functions in metabolic homeostasis (92). Sestrin1 and Sestrin2 activate AMPK through direct interaction with the α subunit of the AMPK complex (66). Sestrin2 acts by activating AMPK and inhibiting various mechanisms of mTORC1. We know that AMPK and mTORC1 play critical roles in metabolism, and Sestrin2 is involved in many biological processes as an upstream regulator of AMPK and mTORC1 kinases (57) (**Figure 1**). Previous experiments in liver-specific *Sesn3* transgenic mice and knockout mice showed that the transgenic mice were protected against insulin resistance induced by a high-fat diet, while the *Sesn3* knockout mice showed metabolic defects such as insulin resistance and glucose intolerance (93, 94). Therefore, we can recognize that Sestrin2 is a potential insulin sensitizer, and that Sestrin deficiency and/or dysfunction may lead to insulin resistance, which can lead to the development of diabetes. Sestrin2 may be a potential therapeutic target for metabolic diseases such as diabetes (82).

Sestrin2 and Non-Alcoholic Fatty Liver Disease

With the global trend in obesity and its related metabolic syndromes, non-alcoholic fatty liver disease (NAFLD) has become an important cause of chronic liver disease in developed countries (95). NAFLD is the hepatic manifestation of metabolic syndrome characterized by intracellular excessive accumulation of lipids in hepatocytes, excluding alcohol and other damaging factors (95). NAFLD involves a range of liver pathological changes, including steatosis, steatohepatitis, advanced fibrosis, and cirrhosis (96). Existing studies have shown that NAFLD is closely associated with persistent ER stress, inhibition of autophagy, mitochondrial dysfunction, insulin resistance, lipotoxicity, and overnutrition (15, 71, 96, 97). Overnutrition and obesity give rise to excessive lipid accumulation in hepatocytes, known as hepatic steatosis (98). We have previously shown that overnutrition can lead to chronic mTORC1 activation (53). mTORC1 can intensify the transcriptional activity of sterol regulatory element binding protein (SREBP) and the expression of lipogenic genes to enhance lipid synthesis (**Figure 1**). It is evident that chronic mTORC1 activation along with persistent inhibition of autophagy attenuates clearance of liver lipid droplets, ultimately leading to hepatosteatosis (99). As a feedback

inhibitor of mTORC1, Sestrin2 can partially ease the effect of chronic mTORC1 activation. For instance, loss of dSesn leads to moderate downregulation of AMPK and upregulation of dTORC1 in *Drosophila*, which contributes to the increased expression of liposomal enzyme genes and ultimately to the accumulation of triglycerides (24). Similarly, a study has confirmed that hepatosteatosis is more serious, and that the primary cause of hepatosteatosis is reduced lipid β-oxidation due to reduced autophagy or mitochondrial biogenesis, rather than increased adipogenesis in Sestrin2-deficient liver (57). Furthermore, Sestrin2 also reduces the susceptibility of the liver to oxidative damage *via* the NRF2/KEAP1 signaling pathway (43). In mice with Sestrin2 deficiency, cells continue to translate large amounts of proteins during ER stress, which subsequently leads to extensive liver damage, inflammation, and fibrosis (15). Accordingly, once induced by ER stress, Sestrin2 maintains endoplasmic reticulum homeostasis by inhibiting the AMPK/mTORC1 signaling pathway (**Figure 1**), thereby protecting against hepatosteatosis (15). Kim et al. found that carbon monoxide can induce Sestrin2 upregulation, and Sestrin2 protects against hepatosteatosis by activating autophagy through the AMPK/mTORC1 axis in a cellular model of NAFLD (48). More interestingly, Sestrin2 plays an important role in the protection against lipotoxicity-associated oxidative stress in the liver *via* suppression of JNKs (72). In summary, Sestrin2 has a significant impact on lipid metabolism and represents a potential therapeutic strategy for NAFLD.

Sestrin2 and Myocardial Ischemia/ Reperfusion Injury

Coronary heart disease, also known as ischemic heart disease (IHD), refers to the interruption of blood flow to the heart muscle due to atherosclerosis, coronary thrombosis, and narrowing of the small arteries of the heart, which remains the leading cause of death worldwide because the incidence of IHD increases with age (100, 101). After an acute myocardial infarction, although early and successful myocardial reperfusion through thrombolytic or percutaneous coronary intervention is the most effective way to rescue the ischemic heart and improve the clinical outcome, the recovery of blood flow can result in myocardial injury, which reduces the efficacy of myocardial reperfusion, namely ischemia/ reperfusion (I/R) injury (78, 102). Myocardial I/R injury is closely related to ROS, calcium overload, energy metabolism disorders, acidosis, and inflammation (102). Some studies have reported that the I/R process usually results in elevated levels of ROS production, especially in the early stages of reperfusion, directly causing myocardial injury (103). Moreover, excessive ROS leads to programmed cell death through the activation of the mitogen-activated protein kinase signaling pathway (104). Mitochondria have an important role in ROS degradation, and dysfunctional mitochondria are the main sources of pathological ROS (105, 106). AMPK can protect mitochondria and play an antioxidant role during the I/R process (78). Furthermore, AMPK has an essential role in the activation of glucose uptake in the ischemic heart (107–109). AMPK also activates 6-phosphofructo 2-kinase, which leads to the production of fructose 2, 6-bisphosphate, further promoting glucose utilization in the ischemic heart (75, 110, 111). Therefore, AMPK is a protein kinase with significant cardiac protection against myocardial I/R injury (74). Sestrin2 has been shown to increase the activation of AMPK *via* interactions with LKB1 to improve myocardial substrate metabolism under I/R stress (75). Sestrin2 was originally characterized as a critical antioxidant protein that contributes to cycling of peroxiredoxins (77). Independent of this redox-regulating activity, Sestrin2 can modulate the activation of AMPK to maintain the integrity of mitochondrial function and reduce the generation of ROS (14, 66, 74, 112). A study by Quan et al. (76) revealed that Sestrin2 greatly reduces myocardial damage by modulating inflammation and redox homeostasis in mouse hearts during I/R stress. Hence, Sestrin2 provides cardioprotection by repressing ROS during I/R injury. Furthermore, Quan et al. (73) found that the decreased Sestrin2 levels in aging and *Sesn2*-knockout mice led to increased sensitivity to ischemic insults and areas of myocardial injury, which aggravated worsened cardiac dysfunction. Sestrin2 protects mitochondrial function by activating the AMPK/PGC-1α signaling pathway during myocardial ischemia (73). Sestrin2 has been shown to be upregulated under anoxic and ischemic conditions and has a protective role against myocardial ischemia (7, 74, 78). The loss of Sestrin2 aggravates the accumulation of fatty acids, thereby altering substrate metabolism in the heart and increasing the production of ROS (37, 78). Inactivation of the *SESN* gene in invertebrates can lead to a variety of metabolic diseases such as muscle degeneration, oxidative damage, fat accumulation, and mitochondrial dysfunction (4). Existing studies have reported that Sestrin2 is involved in the protection of cardiovascular disease by regulating the AMPK signaling pathway (38). Sestrin2 protein accumulates in the heart during myocardial ischemia (17), and the myocardial infarction area in *Sesn2* knockout mice was significantly larger than that in wild-type mice when myocardial ischemia reperfusion occurred (74). In conclusion, Sestrin2 has an influential role in cardioprotection during myocardial I/R injury. Therefore, Sestrin2 may be a therapeutic target for cardiovascular disease, potentially revealing a new avenue of investigation for the treatment of cardiovascular diseases.

PROBLEMS AND PROSPECTS

Sestrin2 is a critical intracellular sensor that activates AMPK and inhibits mTORC1 to regulate autophagy, ER stress, inflammation, metabolic homeostasis, and oxidative stress. Clearly, the AMPK/ mTORC1 axis is regulated by Sestrin2 and it provides the main channel for its function. Sestrin2 regulates metabolism-related signaling pathways, as summarized in **Figure 1**. However, despite their physiological relevance, the exact mechanism by which Sestrin2 promotes AMPK activation remains unclear. Therefore, further studies are needed to determine the detailed molecular function of Sestrin2.

Evidence suggests that Sestrin2 has an important clinical function in responding to a variety of metabolic diseases, such as diabetes mellitus, insulin resistance, and lipid metabolism

disorders. In recent studies, serum Sestrin2 levels were significantly reduced in obese children and patients with diabetic nephropathy (113, 114). This suggests that the expression or secretion of Sestrin2 is somewhat obstructed in the disease state. Furthermore, a study by Kim et al. revealed that in NAFLD cell models, carbon monoxide protects the liver against steatosis by inducing upregulation of Sestrin2, which activates autophagy through the AMPK/mTORC1 axis (48). Consistent with this view, as a glucagon-like peptide 1 (GLP-1) analog, liraglutide could reverse NAFLD by enhancing the level of Sestrin2 protein and the Sestrin2-mediated NRF2/HO-1 pathway (71). Therefore, we hypothesized that upregulation of Sestrin2 expression could ameliorate metabolism-related diseases. Sestrin2 shows great potential as a good prognostic marker and a viable therapeutic target in a variety of diseases. However, how to induce Sestrin2 upregulation remains elusive under different disease conditions. To design therapeutic strategies to upregulate Sestrin2, it is important to further study the upstream and downstream pathways of the multipotent beneficial effects of Sestrin2. Future studies should use transgenic animal models with conditional organ-specific knockout of *Sesn2* and attempt to link Sestrin2 levels to disease progression, which will help us identify biochemical pathways regulated by Sestrin2 in specific diseases.

AUTHOR CONTRIBUTIONS

LG and ZZW worked out the theme and content of the article. ZGW completed the production of charts. ZZ reviewed and revised the full text. All authors contributed to the article and approved the submitted version.

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MIA SH3 Domain ER Export Factor 3 Deficiency Prevents Neointimal Formation by Restoring BAT-Like PVAT and Decreasing VSMC Proliferation and Migration

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Abnormal proliferation and migration of vascular smooth muscle cells (VSMCs) and excessive accumulation of dysfunctional PVAT are hallmarks of pathogenesis after angioplasty. Recent genome-wide association studies reveal that single-nucleotide polymorphism (SNP) in MIA3 is associated with atherosclerosis-relevant VSMC phenotypes. However, the role of MIA3 in the vascular remodeling response to injury remains unknown. Here, we found that expression of MIA3 is increased in proliferative VSMCs and knockdown of MIA3 reduces VSMCs proliferation, migration, and inflammation, whereas MIA3 overexpression promoted VSMC migration and proliferation. Moreover, knockdown of MIA3 ameliorates femoral artery wire injury-induced neointimal hyperplasia and increases brown-like perivascular adipocytes. Collectively, the data suggest that MIA3 deficiency prevents neointimal formation by decreasing VSMC proliferation, migration, and inflammation and maintaining BAT-like perivascular adipocytes in PVAT during injury-induced vascular remodeling, which provide a potential therapeutic target for preventing neointimal hyperplasia in proliferative vascular diseases.

Keywords: PVAT, MIA3, VSMCs, neointima, adipocytes

INTRODUCTION

Coronary stents are routinely placed in the treatment of coronary artery disease (CAD). Though application of drug-eluting stents (DES) that release antiproliferative drugs, such as paclitaxel-eluting stents and sirolimus-eluting stents, has dramatically increased success rate compared to regular bare-metal stents (BMS), in-stent re-stenosis (ISR) remains to be problematic in coronary interventional treatment (1, 2).

The underlying molecular mechanism of in-stent neointimal formation has not been fully understood. VSMCs are the major cell types of the medial layer arteries and normally adopt a quiescent, contractile phenotype to regulate vascular tone. However, VSMCs retain phenotypic plasticity and can dedifferentiate into a proliferative, synthetic state, which associated with altered contractile marker expression such as smooth muscle myosin II (SM-myosin II), smoothelin, calponin, and smooth muscle-actin (3–5). Vascular injury caused by angioplasty, stenting, or bypass surgery triggers phenotypic switch of vascular smooth muscle cells (VSMCs) and subsequent abnormal proliferation and migration of VSMCs, leading to excessive formation of neointima, which contributes to occlusive vascular diseases such as atherosclerosis, intimal hyperplasia associated with restenosis, and vein graft stenosis (6–9). Therefore, unraveling the molecular mechanisms involved in regulating VSMC phenotypic switch, proliferation, and migration is a vital step toward understanding the pathology of restenosis.

Adipose tissues are present at multiple locations in the body. Most blood vessels are surrounded with adipose tissue, which is referred to as perivascular adipose tissue (PVAT). PVAT shows characteristics of beige adipose tissue (BeAT) in human and brown adipose tissue (BAT)-like in mice. PVAT is not always BAT in mice and humans. It depends on the anatomic location and environmental/metabolic context. While endovascular injury originates in the endothelium, its impact is felt throughout the blood vessel wall, including the adventitia and perivascular adipocytes (10). Phenotypic changes in PVAT after vascular injury promote release of adipocytokines that can regulate inflammation, VSMC proliferation, and neovascularization, thereby contributing to neointimal formation.

MIA3 (also named TANGO1) protein is localized to the endoplasmic reticulum exit site, where it loads cargo molecules, such as collagen VII, into COPII carriers to promote their secretion out of the endoplasmic reticulum (11, 12). Several large-scale meta-genome-wide association studies (GWAS) identified significant association between SNP rs17465637 in the MIA3/TANGO1 gene and CAD in the European ancestry populations and also Chinese populations (13, 14). A recent GAWS for 12 atherosclerosis-relevant phenotypes identified that the risk of single-nucleotide polymorphism (SNP) rs67180937 in the chromosome 1q41 locus was associated with lower VSMC MIA3 expression and lower proliferation (15). However, the role of MIA3 during neointimal formation is unknown.

Here, the present study showed that MIA3 was upregulated during SMC phenotypic modulation, which was induced by FBS administration. Conversely, the knockdown of MIA3 expression led to impaired proliferation and migration of SMCs, while MIA3 overexpression induced SMC proliferation. Knockdown of MIA3 ameliorates femoral artery wire injury-induced neointimal hyperplasia and increases brown-like perivascular adipocytes. This preliminary study provides new insights into the role and molecular mechanisms of MIA3 controlling function of VSMC and PVAT and identifies a novel potential target for suppression of neointimal formation.The authors declare that all supporting data are available within the article and its online supplementary file.

Murine Model of Femoral Artery Wire Injury

Femoral artery wire injury was established in male C57BL/6 mice, as previously described (16). Briefly, mice were subjected to left femoral artery injury using a diameter angioplasty guidewire (0.35-mm diameter; Cook Inc, Bloomington, IN) under ketamine HCl (100 mg/kg, intraperitoneal injection) and xylazine HCl (10 mg/kg, intraperitoneal injection) anesthesia and aseptic conditions. Wire-injured femoral arteries were harvested at 28 days postsurgery, fixed with 4% paraformaldehyde, embedded in paraffin wax, and sectioned at 8-μm intervals for histology analysis. The cross-sections of the injured arteries were obtained at 500 to 1500 μm distant from the ligation at 100-μm intervals.

Sections were stained with hematoxylin and eosin (Servicebio, China) and Masson trichrome (Servicebio, China) stain kit and the images were acquired with Leica DM750 microscope. Five levels of hematoxylin and eosin staining images at 200-μm intervals were used for quantification of neointimal formation per mice. Measurements were made for lumen circumference, the internal elastic lamina circumference, and the circumference of the external elastic lamina. Measurements were quantified using ImageJ software (National Institutes of Health, Maryland, USA). The neointimal area was determined by subtracting the luminal area from the area bound by the internal elastic lamina. The media area was determined by subtracting the area bound by the internal elastic lamina from the area bound by the external elastic lamina. The intima-to-media ratio was determined by the intimal area divided by the medial area. Percentage of stenosis was calculated as the ratio of the intimal area to the area inside the original internal elastic lamina. Measurements were performed with the observer blinded to experimental group.

The animal procedures were performed in accordance with the Institutional Animal Care and Use of Laboratory Animals and were approved by the Animal Care Committee of Shanghai Jiao Tong University.

Histology

The tissue sections were immersed in sodium citrate buffer (10 mM, pH 6.0) and heat retrieved for 20 min in a 100°C water bath to perform antigen retrieval. The slices were permeabilized and blocked in PBS-T (0.02% Triton X-100) with 1% goat serum for 1 h. Immunostaining was performed using the following antibodies diluted in PBS-T (0.02% Triton X-100) at 4°C overnight: anti-MIA3 (1:200, 17481-1-AP; WUHAN SANYING; China), Rabbit anti-Ki67 (1:200, Abcam, ab16667), Goat anti-Perilipin-1 (1:200, Abcam, ab61682), and Rabbit anti-UCP1 (1:200, Abcam, ab234430). Slides were mounted with Vectashield mounting medium containing DAPI (Vector Laboratories, Burlingame, CA, USA). After washing with PBS, sections were incubated with secondary antibodies (488 nm conjugated anti-rabbit or goat secondary antibody and 594 nm conjugated anti-rabbit) diluted in PBS-T (0.02% Triton X-100) for 1 h at room temperature. Following wash with PBS for three times, slides were mounted with

vectashield mounting medium containing DAPI (Vector Laboratories, Burlingame, CA, USA) and imaged using fluorescent microscope.

Cell Culture

Human aortic VSMCs were purchased from ATCC company (PCS-100-012, USA) and cultured in Vascular Smooth Muscle Cell Growth Kit (PCS-100-042, USA) supplemented with 10% FBS (Hyclone, USA).

Knockdown of MIA3 *In Vitro*

The siRNA were purchased from Genepharma company and transfected into cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) for knockdown of MIA3.The sequence of the MIA3 oligos was 5'-CCAGGUAGUUCAUGAAUAU-3'.

Overexpression of MIA3 *In Vitro*

The human MIA3 full-length cDNA was subcloned into pCMV3 vector and transfected into cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) for knockdown of MIA3. The plasmid is confirmed by full-length sequencing.

Real-Time Quantitative–Polymerase Chain Reaction

Real-time polymerase chain reaction was performed according to our laboratory workflow. The expression of the involved genes was normalized to GAPDH, and experiment was repeated in triplicate. The primer sequences were listed as follows (forward, reverse): MIA3-homo: 5'-AAGTTCCAACAGATGAGACGGA-3', 5'-GGTTCAGGTTCCCTTTCCTTAG-3'; α-SMA-homo: 5'-GAGAGGAGCAAAATCTGTCCG-3', 5'-GGGGGAATTATCTTTCCTGGTCC-3'; Cyclin D1-homo: 5'-TGGAGCCCGTGAAAAAGAGC-3', 5'-TCTCCTTCATCTTAGAGGCCAC-3'; IL-1β-homo: 5'-AGCTACGAATCTCCGACCAC-3', 5'-CGTTATCCCATGTGTCGAAGAA-3'; IL18-homo: 5'-TCTTCATTGACCAAGGAAATCGG-3', 5'-TCCGGGGTGCATTATCTCTAC-3'; CCL7-homo: 5'-TGCTCAGCCAGTTGGGATTA-3', 5'-GGACAGTGGCTACTGGTGGT-3'; CxCL8-homo: 5'-TTTTGCCAAGGAGTGCTAAAGA-3', 5'-AACCCTCTGCACCCAGTTTTC-3'; GAPDH-homo: 5'-CACCAGGGCTGCTTTTAACT-3', 5'-TGGGATTTCCATTGATGACA-3'; MIA3-mus: 5'-GTGAGGATGAAGGTGACGA-3', 5'-CTTGCTACCCTGAAGACGA-3'; GAPDH-mus: 5'-TGTTTCCTCGTCCCGTAGA-3', 5'-ATCTCCACTTTGCCACTGC-3'.

Western Blot Analysis

We used RIPA Lysis Buffer (P0013C; Beyotime Biotechnology, China) to extract protein from SMCs. Twenty micrograms of protein was loaded in 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel. Antibodies used were as follows: anti-MIA3 (1:1,000, 17481-1-AP; WUHAN SANYING; China), anti-α-SMA (1:1,000, ab7817; Abcam; USA), and anti-Cyclin D (1:1,000, ab16663; Abcam; USA). A horseradish peroxidase-conjugated goat anti–rabbit secondary antibody (1:1,000, A0277; Beyotime Biotechnology, China) was then added to the membranes at room temperature for 2 h. Subsequently, ImageJ analysis software was used to quantify the bands of Western blot images, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal reference. Each experiment was repeated three times.

Immunofluorescence Staining

Cells were seeded on glass coverslips placed in 24-well plates and fixed with 4% paraformaldehyde for 15 min and then permeabilized with 0.25% Triton X-100 in PBS for 10 min. Then, cells were washed three times with PBS and blocked with 5% goat serum in PBS for 30 min. Next, the primary antibodies were used to incubate VSMCs overnight at 4°C. Appropriate secondary antibodies were incubated with VSMCs for 1 h at room temperature. DAPI was used to stain the nuclei. The images were acquired using a fluorescent microscope (Zeiss LSM780, Carl Zeiss).

Cell Proliferation Assay

Cell viability was measured using the cell counting, MTT assay, and EdU staining assay. In the cell counting assay, VSMCs transfected with MIA3 siRNA or overexpressing vector and scramble siRNA or control empty vector were seeded at an initial density of 2×10^4 per well in a 12-well plate in VSMC culture medium with 10% FBS, and the cells were harvested and counted at the designated time points. For MTT assay, VSMCs were seeded in a 96-well plate in VSMC culture medium with 10% FBS, and 50 μl of MTT solution [5 mg/ml; A600799, Sangon Biotech (Shanghai) Co., Ltd.] was added to each well and incubated for 4 h at the designated time points. To dissolve the formazan crystals, 80 μl of mixture of 40 ml of isopropanol plus 44 μl of 37% HCl was added to each well after the media were removed. Absorbance was measured at 570 nm using a microplate reader (Bio-Rad). EdU staining assay was performed using the EdU Staining Proliferation Kit (iFluor 647) according to the protocol booklet. Briefly, equal VSMCs were seeded in a six-well plate after starved for 24 h and allowed to grow in VSMC with 10% FBS culture medium, and the EdU solution was added to VSMCs for 2 h under growth conditions. VSMCs were fixed in 4% paraformaldehyde, permeated, and stained with reaction mix to fluorescently label EdU for 30 min. The samples were mounted on glass slides and were visualized using an inverted fluorescent microscope (Carl Zeiss, Oberkochen, Germany).

For propidium iodide cell cycle analysis, VSMCs were starved for 24 h and grown in culture medium for 24 h. VSMCs were then harvested and immersed in 70% ethanol at the designated time point. Cell DNA was stained with 50 μg/ml propidium iodide and 20 μg/ml RNase at 37°C for 30 min. Cell cycle was analyzed using a flow cytometry with a FACS canTM flow cytometer (BD Biosciences, Mansfield, MA, USA).

Cell Migration Assay

For the wound healing assay, VSMCs (2×10^5 cells) transfected with MIA3 siRNA or overexpressing vector and scramble siRNA or control empty vector were plated onto six-well plates and serum-starved for 24 h. An artificial wound (scratch) was generated using a 200-μl pipette tip and cultured for 24 h in serum-starved medium. SMCs were visualized using a microscope

and captured images were assessed using Image−Pro Plus software.

For the Transwell migration assay, VSMCs were cultured on the microporous membrane (8.0 μm) in the upper chamber of the Transwell (Costar 3422; Corning Incorporated, NY, USA) in serum-free medium for 12 h, and VSMC culture medium with 10% FBS was added into the lower chamber. After 24 h, SMCs were allowed to migrate from the upper chamber to the underside of the membrane. The unmigrated cells in the upper chamber were gently removed using a cotton swab. Cells migrated through the membrane to the lower chamber were fixed in paraformaldehyde and stained with 0.05% crystal violet. Migrated SMCs on the lower membrane were counted using an Olympus light microscope and analyzed using the Image J software.

Lentivirus Generation and Transduction

Lentivirus was produced using pLV10N-U6-shRNA vector and mouse MIA3 shRNA was cloned into the vector, and generated in the 293T viral packaging cell line. Femoral arteries were transduced locally with 10^9 IU per mouse in the presence of 10 μg/ml DEAE-dextran after wire injury. The efficiency of MIA3 knockdown was estimated by real-time quantitative-polymerase chain reaction. The sequence of MIA3 shRNA was GCAACCAGACTGGTCACTTCA.

RNA Extraction and RNA High-Throughput Sequencing

RNA-sequencing was conducted on MIA3 knockdown and control scramble human aortic smooth muscle cells (HASMCs). Total RNA was extracted using TRIzol (Invitrogen, USA) following the manufacturer's instructions. The total RNA concentration, the RIN value, 28S/18S, and the fragment size were measured using an Agilent 2100 Bioanalyzer (Agilent, USA). Oligo(dT)-attached magnetic beads were used to purify mRNA. The BGISEQ-500 (Shenzhen Huada Gene, China) platform was used for high-throughput sequencing to obtain a 50-bp sequencing read. The raw data were subjected to quality control to obtain effective reads. SOAPnuke (v 1.5.2) and Trimmomatic (v0.36) were used to perform statistical analysis and filter out reads of low to moderate quality, polluted connectors, and unknown nucleotides with high N content before data analysis to ensure reliability. The clean reads were mapped to the reference genome using HISAT2 (v2.0.4). Ericscript (v0.5.5) and rMATS (V3.2.5) were used to fusion genes and differential splicing genes (DSGs).

Statistical Analysis

All values in the graphs represent the mean ± SEM. Comparison between two groups were compared using an unpaired Student's *t*-test. Statistical analyses were performed in SPSS version 13.0 (SPSS, Inc., Chicago, IL). $p < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

MIA3 Expression Is Increased in Proliferative Vascular Smooth Muscle Cells

FBS is a potent mediator of the SMC phenotypic modulation from a contractile to a synthetic state by promoting SMC proliferation as well as repressing SMC marker gene expression. To investigate whether MIA3 is associated with VSMC proliferation, we treated the cultured HASMC with FBS, and our data revealed that MIA3 is expressed in VSMCs and the mRNA and protein level of MIA3 in cultured proliferating HASMCs are significantly increased compared with the serum-starved quiescent HASMCs, whereas SMC contractile gene α-SMA was significantly reduced (**Figures 1A–C**). Proliferation gene Cyclin D expression was enhanced in parallel with MIA3 expression. Immunofluorescence staining showed that the expression of MIA3 in cultured HASMCs was induced by FBS (**Figure 1D**). These data suggest that upregulation of MIA3 is positively correlated with the synthetic SMC phenotype and MIA3 may regulate VSMC proliferation.

As FBS-induced VSMC proliferation contributes to neointimal hyperplasia, we detected MIA3 expression changes during neointimal formation in a mouse femoral artery wire injury model. Compared with the control sham group, MIA3 mRNA level in femoral arteries at 28 days after wire injury was markedly increased (**Figure 1E**). Consistent with quantitative real-time reserve transcription polymerase chain reaction, MIA3 protein expression was increased in the femoral arteries at 28 days after wire injury by immunofluorescent staining (**Figure 1F**). Taken together, our results demonstrate that the increase of MIA3 in the femoral arteries after wire injury may be involved in neointimal hyperplasia

Knockdown of MIA3 Inhibits Vascular Smooth Muscle Cell Proliferation *In Vitro*

Proliferation of VSMCs plays a vital role in the development of neointimal formation. To investigate whether MIA3 participated in the proliferation of VSMCs, we used siRNA targeting MIA3 to silence its expression in VSMCs. As shown in **Figures 2A, B**, significantly reduced expression of MIA3 was observed in HASMCs infected with MIA3 siRNA compared with control scramble siRNA. As expected, cell counting, MTT assay, and EdU incorporation assay showed that MIA3 knockdown attenuated VSMC proliferation (**Figures 2C–E**). Furthermore, flow cytometry analysis of cell cycle status showed that MIA3 knockdown caused a significant increase in the percentage of cells in G1 phase (from 42.1% ± 2.0% to 46.1% ± 1.5% in FBS-stimulated cells) but decreased the percentage of cells in S phase (from 42.2% ± 0.4% to 35.1% ± 1.6% in FBS-stimulated cells) (**Figure 2F**). Collectively, these results demonstrate that knockdown of MIA3 attenuates VSMC proliferation *in vitro*.

Knockdown of MIA3 Inhibits Vascular Smooth Muscle Cell Migration *In Vitro*

VSMC migration from the medial layer is another key event to build neointima. Wound healing and Boyden chamber assay

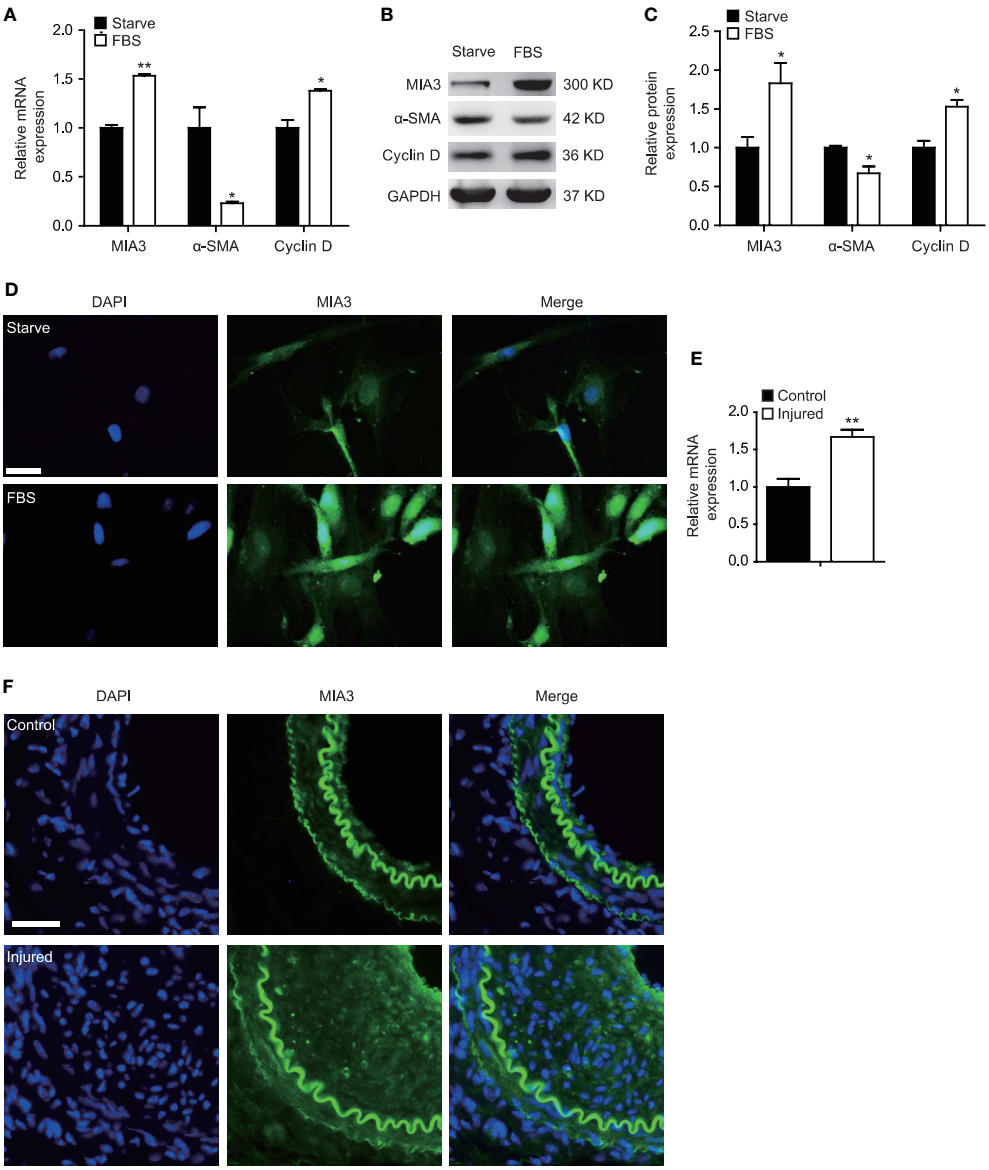

FIGURE 1 | MIA3 expression is increased in proliferative vascular smooth muscle cells (VSMCs). **(A)** mRNA levels of MIA3, α-SMA (smooth muscle α-actin), and Cyclin D1 by quantitative real-time reverse transcription polymerase chain reaction in VSMCs after treatment with medium containing 10% fetal bovine serum for 24 h and the control FBS-starve VSMCs. Data are represented as the mean ± SEM, $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$, *t*-test. **(B)** At 48 h after FBS treatment, VSMC extracts were collected for determining the protein levels of MIA3, α-SMA (smooth muscle α-actin) and Cyclin D1 by Western blotting. GAPDH served as loading control expression. **(C)** Quantification of protein expression normalized to the levels of GAPDH. Data are represented as the mean ± SEM, $n = 3$. $^{*}p < 0.05$, *t*-test. **(D)** Immunofluorescent staining of MIA3 in VSMCs after treatment with medium containing 10% fetal bovine serum for 24 h and the control FBS-starve VSMCs. DAPI represents indicates 4',6-diamidino-2-phenylindole throughout the article; scale bar: 50 μm. **(E)** mRNA levels of MIA3 by quantitative real-time reserve transcription polymerase chain reaction in femoral artery after wire injury for 28 days. Data are represented as the mean ± SEM, $n = 3$. $^{**}p < 0.01$, *t*-test. **(F)** Immunofluorescent staining of MIA3 in the femoral artery following wire injury for 28 days; scale bar: 100 μm.

were used to test the role of MIA3 in the VSMC migration. Consistent with the role of MIA3 in VSMC proliferation, MIA3-specific siRNA efficiently attenuated VSMC migration by wound healing (**Figure 3A**) and Boyden chamber assay (**Figure 3B**). The above data suggested that MIA3 knockdown in VSMC decreases cell migration *in vitro*.

Overexpression of MIA3 Promotes Vascular Smooth Muscle Cell Proliferation and Migration *In Vitro*

Our above data showed that knockdown of MIA3 blocked VSMC proliferation and migration. We next investigated the effect of MIA3 overexpression on VSMC proliferation and migration. The

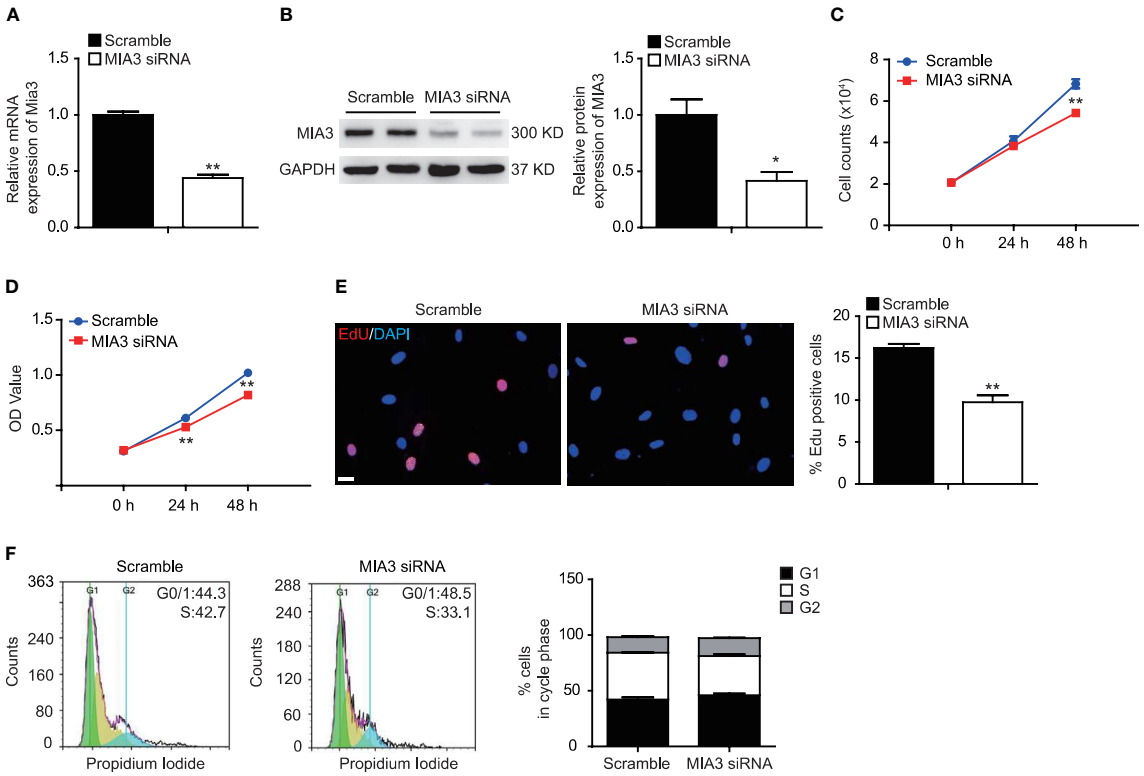

FIGURE 2 | Knockdown of MIA3 inhibits vascular smooth muscle cell (VSMC) proliferation. **(A)** MIA3 mRNA levels in human aortic smooth muscle cells (HASMCs) with small interfering RNA (siRNA) MIA3 and scramble siRNA. Data are represented as the mean ± SEM, $n = 3$. $^{**}p < 0.01$, *t*-test. **(B)** MIA3/TANGO1 protein levels in human aortic smooth muscle cells (HASMCs) with small interfering RNA (siRNA) MIA3 and scramble siRNA with quantitative data at right. GAPDH served as loading control expression. Data are represented as the mean ± SEM, $n = 3$. $^{*}p < 0.05$, *t*-test. **(C, D)** VSMC proliferation was determined by cell counts **(C)** and 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay **(D)** in VSMCs with or without transfection of MIA3-specific siRNAs. All data were indicated as the means ± SEM, $n = 3$. $^{**}p < 0.01$, *t*-test. **(E)** VSMC proliferation was determined by 5-ethynyl-2'deoxyuridine (EdU) incorporation in VSMCs with or without transfection of MIA3-specific siRNAs. Percentage of EdU staining-positive cells was quantified on the right. Scale bar: 50 μm. Data are represented as the mean ± SEM, $n = 3$. $^{**}p < 0.01$, *t*-test. **(F)** VSMC proliferation was determined by PI-cell cycle analysis in VSMCs with or without transfection of MIA3/TANGO1-specific siRNAs. Data are represented as the mean ± SEM, $n = 3$, *t-test*.

efficiency of Foxp1 overexpression was confirmed by RT-qPCR and Western blot (**Figures 4A, B**). Importantly, VSMC proliferation was increased upon overexpression of MIA3 determined by cell counting, MTT assay, and EdU incorporation assay (**Figures 4C–E**). The wound healing and Boyden chamber assay further demonstrated that MIA3 overexpression increased VSMC migration (**Figures 4F, G**). Together, upregulation of MIA3 itself promotes cell proliferation and migration of VSMCs.

Knockdown of MIA3 Attenuates Femoral Artery Wire Injury–Induced Neointimal Formation in Mice

To investigate the function of MIA3 in femoral artery wire injury–induced neointimal formation in mice, lentiviral control shRNA or lentiviral MIA3 shRNA was perivascularly applied to femoral arteries immediately after wire injury, as described in the previous studies (16, 17). The decrease of MIA3 expression in wire-injured femoral artery applied with lentiviral MIA3 shRNA was confirmed by real-time quantitative–polymerase chain reaction (**Figure 5A**). Consequently, at 28 days after femoral artery wire injury, knockdown of MIA3 significantly attenuated neointimal formation, as shown by the decreased neointimal area, neointima/media ratio, and percentage of stenosis in the lentiviral MIA3 shRNA-treated injured artery, but has no influence on vessel media area (**Figures 5B, C**). Masson trichrome staining showed that knockdown of MIA3 significantly reduced ECM deposition after vascular injury (**Figure 5D**). Immunostaining showed a significant reduction of Ki67-positive cells (**Figure 5E**) in the neointima of MIA3 knockdown mice, suggesting that knockdown of MIA3 inhibits the cell proliferation contributing to decreased neointimal hyperplasia after wire injury of femoral artery. These results indicate that MIA3 knockdown markedly suppresses neointimal formation in mice.

Knockdown of MIA3 Maintains BAT-Like Perivascular Adipocytes in PVAT

Extensive inflammatory cell infiltration around the vasculature in response to vascular injury induces histological and phenotypic changes of perivascular adipocytes. Dysfunctional

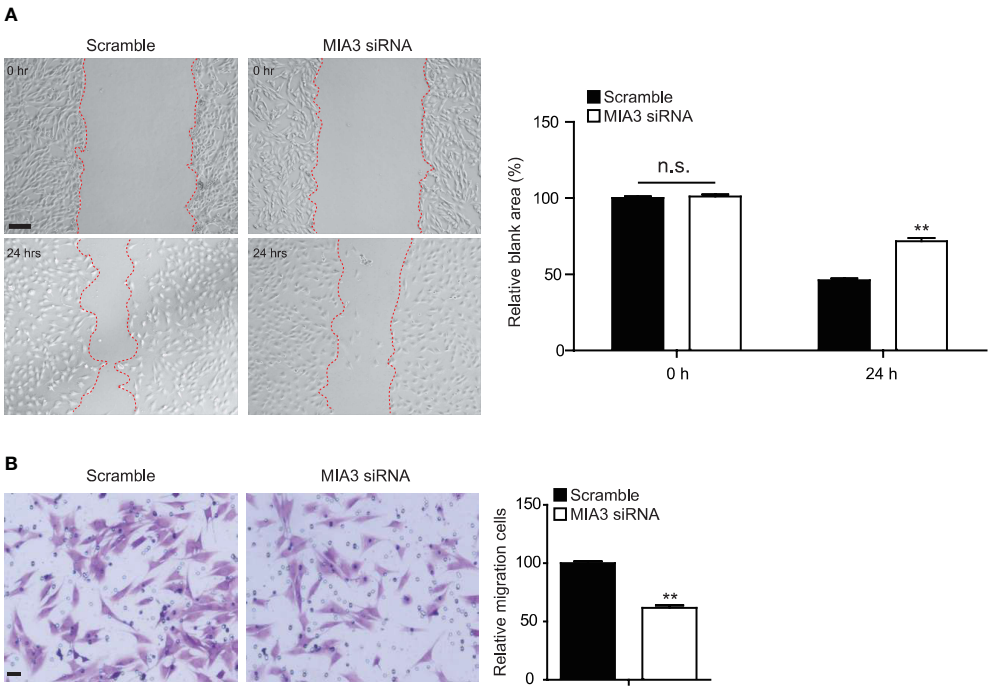

FIGURE 3 | Knockdown of MIA3 inhibits vascular smooth muscle cell (VSMC) migration. **(A)** The wound-induced cell migration assay was performed in VSMCs with or without transfection of MIA3-specific small interfering RNA. The relative blank wound areas in the left were quantified on the right. Scale bar: 100 μm. Data are represented as the mean ± SEM, $n = 3$. n.s. indicates nonsignificant. $^{**}p < 0.01$, *t*-test. **(B)** The transwell assay in VSMCs with MIA3 siRNA or scramble siRNA. The transferred and stained cells were counted on the right. Scale bar: 50 μm. Data are represented as the mean ± SEM, $n = 3$. *t*-test.

PVAT secretes disease-promoting factors that exacerbate pathogenesis of neointimal formation. So, we investigated whether MIA3 deficiency altered PVAT features from BAT-like to WAT-like contributing to neointimal formation. Immunostaining showed a significant increase of UCP-1-positive cells (**Figure 6A**) in the neointima of MIA3 knockdown mice, suggesting that MIA3 deficiency increased brown-like perivascular adipocytes.

PVAT dysfunction is characterized by its inflammatory character, and VSMCs are a significant source of chemokines and cytokines (18). To investigate the mechanism of MIA3 in maintaining BAT-like perivascular adipocytes in PVAT, we performed RNA-sequencing of MIA3 shRNA-treated VSMC and scramble VSMC, and found that knockdown MIA3 in VSMC decreased IL-1β, IL18, CCL7, and CxCL8 expression (Online uploaded excel file), which were confirmed by quantitative real-time reserve transcription polymerase chain reaction (**Figure 6B**). The above results illustrate that knockdown MIA3 in VSMC maintains BAT-like perivascular adipocytes in PVAT *via* inhibiting expression of inflammatory factors.

DISCUSSION

Here, we discovered that MIA3 is a novel regulator in promoting VSMC proliferation and migration during neointimal formation. Knockdown of MIA3 reduces proliferation and migration of SMC. In contrast, MIA3 overexpression promoted VSMC migration and proliferation. Furthermore, administration of MIA3 shRNA lentivirus attenuated femoral artery wire injury-induced neointima in mice. In addition, knockdown MIA3 maintains BAT-like perivascular adipocytes in PVAT *via* inhibiting expression of inflammatory factors following femoral artery wire injury. Our findings identified MIA3 as a novel target for developing antineointima drugs during vascular repair.

VSMCs are the major cell types of medial layer arteries and play a pivotal role in regulating the remodeling process of the vessel wall (19). Fully differentiated SMCs are almost quiescent with little proliferation and are programmed for contraction with relatively high expression of SM myosin heavy chain (SMMHC), SM22a, and calponin. However, in response to local vascular injury, SMCs dedifferentiate from contractile phenotype toward a synthetic state, which was characterized by a decreased expression of contractile SMC marker genes and increased rates of migration and proliferation (19, 20). Subsequent excess proliferation and migration result in an accumulation of synthetic SMCs in the stented artery, which contributes to the in-stent restenosis. Thus, inhibiting the proliferation and migration of intravascular SMC is the predominant therapeutic strategy to prevent the excessive formation of neointima (21, 22).

MIA SH3 Domain ER Export Factor 3 (MIA3) is an evolutionarily conserved endoplasmic reticulum-resident

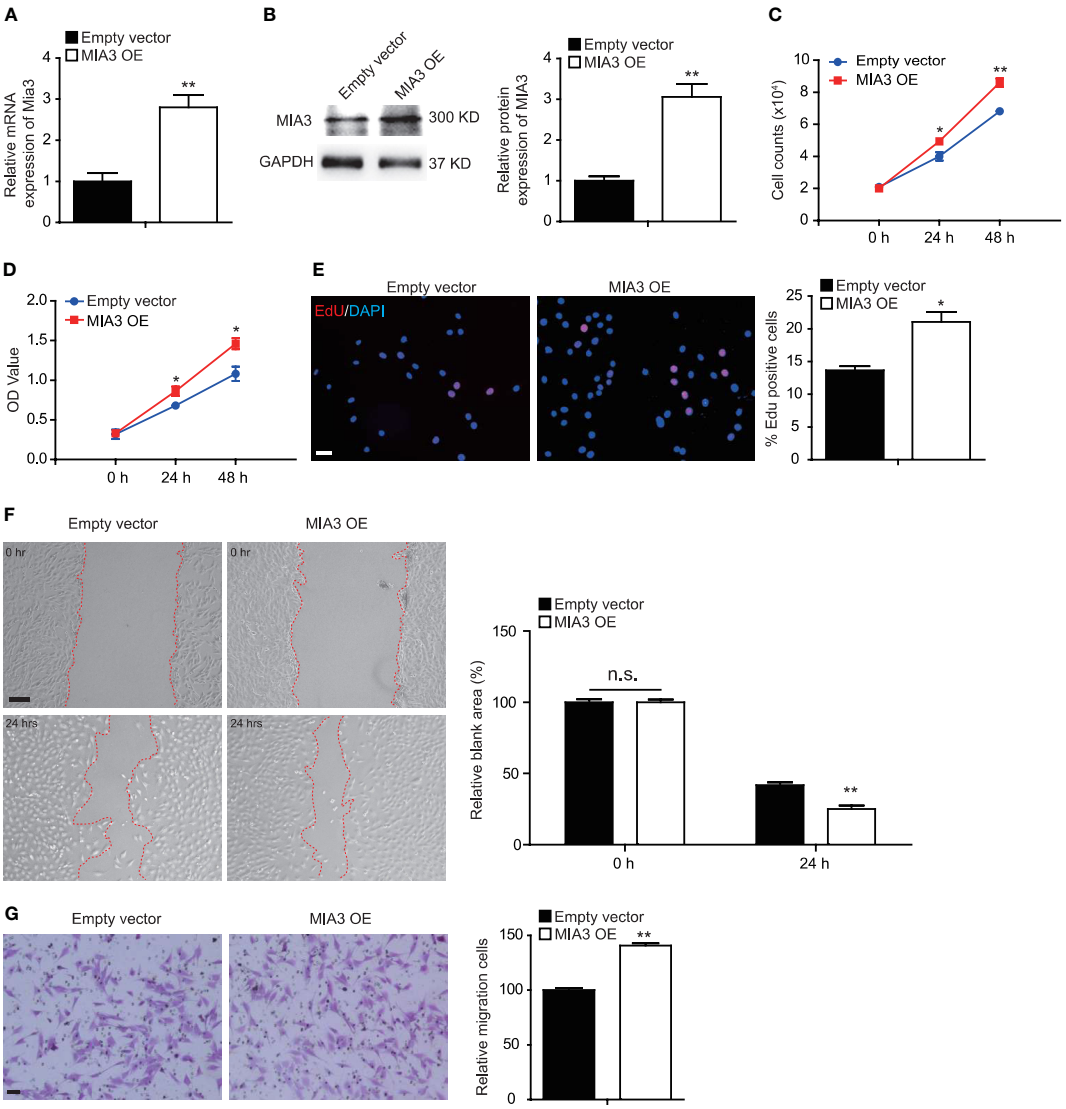

FIGURE 4 | MIA3 overexpression promotes vascular smooth muscle cell (VSMC) proliferation and migration. **(A)** MIA3 mRNA levels in human aortic smooth muscle cells (HASMCs) transfected with MIA3 overexpressing vector (OE) and control empty vector. Data are represented as the mean ± SEM, $n = 3$. $^{**}p < 0.01$, *t*-test. **(B)** MIA3 protein levels in human aortic smooth muscle cells (HASMCs) transfected with MIA3 overexpressing vector and control empty vector with quantitative data on the right. GAPDH served as loading control expression. Data are represented as the mean ± SEM, $n = 3$. $^{*}p < 0.05$, *t*-test. **(C, D)** VSMC proliferation was determined by cell counts **(C)** and 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay **(D)** in VSMCs with or without transfection of MIA3 overexpressing vector. All data were indicated as the means ± SEM, $n = 3$. $^{**}p < 0.01$, *t*-test. **(E)** VSMC proliferation was determined by 5-ethynyl-2'-deoxyuridine (EdU) incorporation in VSMCs with or without transfection of MIA3 overexpressing vector. Percentage of EdU staining–positive cells was quantified on the right. Scale bar: 50 μm. Data are represented as the mean ± SEM, $n = 3$. $^{**}p < 0.01$, *t*-test. **(F)** The wound-induced cell migration assay was performed in VSMCs with or without transfection of MIA3 overexpressing vector. The relative blank wound areas in the left were quantified on the right. Scale bar: 100 μm. Data are represented as the mean ± SEM, $n = 3$. n.s. indicates nonsignificant. $^{**}p < 0.01$, *t*-test. **(G)** The transwell assay in VSMCs transfected with MIA3 overexpressing vector or empty vector. The transferred and stained cells were counted on the right. Scale bar: 50 μm. Data are represented as the mean ± SEM, $n = 3$. $^{**}p < 0.01$, *t*-test.

transmembrane protein and is required for the export of collagen VII (COL7A1) from the endoplasmic reticulum. Mice lacking MIA3 are defective for the secretion of numerous collagens, including collagens I, II, III, IV, VII, and IX, from chondrocytes, fibroblasts, endothelial cells, and mural cells (23). Our study demonstrated that knockdown of MIA3 significantly reduced ECM deposition after vascular injury (**Figure 5D**), indicating that MIA3 may regulate vascular remodeling in response to injury through regulating extracellular matrix secretion.

Genome-wide association studies (GWAS) have described an association between MIA3 rs17465637 A/C polymorphisms and CAD and myocardial infarction (24–26). A recent study

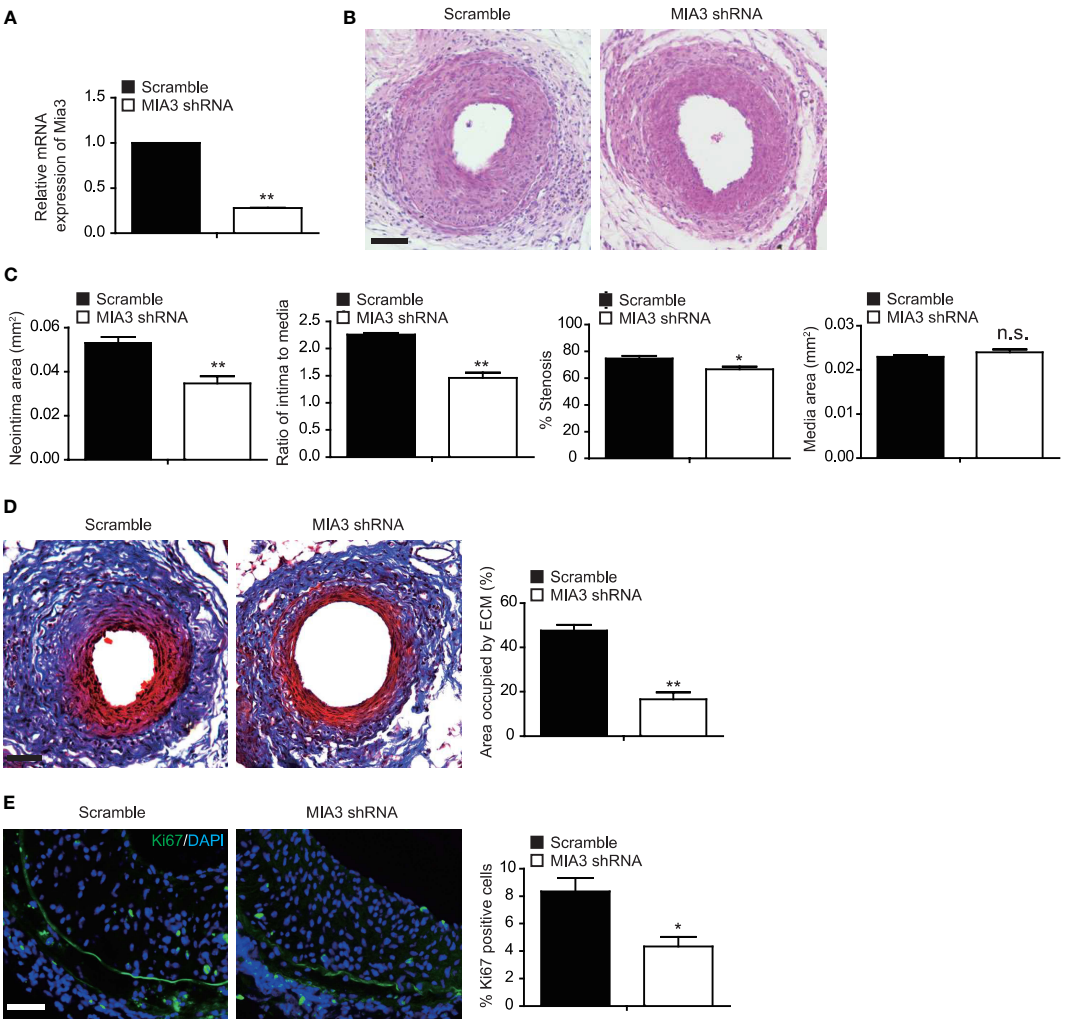

FIGURE 5 | Knockdown MIA3 ameliorates femoral artery wire injury-induced neointimal hyperplasia in mice. **(A)** Quantitative real-time reserve transcription polymerase chain reaction was performed to confirm the decreased expression of MIA3 in the injured femoral arteries. Data are represented as the mean ± SEM, $n = 5$. $^{**}p < 0.01$. *t*-test. **(B)** Representative hematoxylin and eosin (H&E) staining of mouse femoral arteries after wire injury for 28 days from mice infected with MIA3 shRNA lentivirus and scramble shRNA lentivirus. Scale bar: 100 μm. **(C)** The neointima area, intima-to-media ratio, % stenosis, and media area of wire-injured femoral arteries from mice infected with MIA3 shRNA lentivirus and scramble shRNA lentivirus. $^{*}p < 0.05$, $^{**}p < 0.01$, n.s. indicates nonsignificant. $n = 5$, *t*-test. **(D)** Representative Masson trichrome staining of mouse femoral arteries after wire injury for 28 days from mice infected with MIA3 shRNA lentivirus and scramble shRNA lentivirus, with quantification data on the right. Scale bar: 100 μm. Data are represented as the mean ± SEM, $n = 5$. $^{**}p < 0.01$. *t*-test. **(E)** Knockdown MIA3 with shRNA lentivirus exhibit a significant reduction of Ki67-positive cells in the neointima at 28 days after femoral artery wire injury, with representative images (left) and quantification data (right; $n = 5$ for each group). Data are represented as the mean ± SEM, $n = 5$. $^{*}p < 0.05$. *t*-test.

observed a significant reduction of MIA3 protein in VSMCs in thin fibrous caps of late-stage atherosclerotic plaques compared to early fibroatheroma with thick and protective fibrous caps in mice and humans (15), indicating that high MIA3 expression may promote atheroprotective VSMC phenotypic transitions. However, the detailed role of MIA3 in VSMC phenotypic transitions is unclear. FBS is a key stimulus for VSMC proliferation, migration, and phenotypic switch contributing to neointimal formation (27). In this study, the increase of MIA3 in FBS-induced VSMC may contribute to the development of injury-induced neointimal formation. The knockdown of MIA3 results indicated that VSMC proliferation and migration, which are the critical cellular events in vascular neointimal lesion formation, were regulated, at least in part, by MIA3. We showed that local transfer of lentiviral MIA3 shRNA onto the injured arteries could significantly reduce VSMC proliferation and decreased neointimal formation at the 28th day post-injury, providing a basis for preventing or inhibiting in-stent restenosis *via* MIA3 siRNA-coated stents.

A previous study demonstrated that overexpression of endothelial MIA3 significantly increased EC proliferation, migration, and EC tube formation, which suggest that EC-

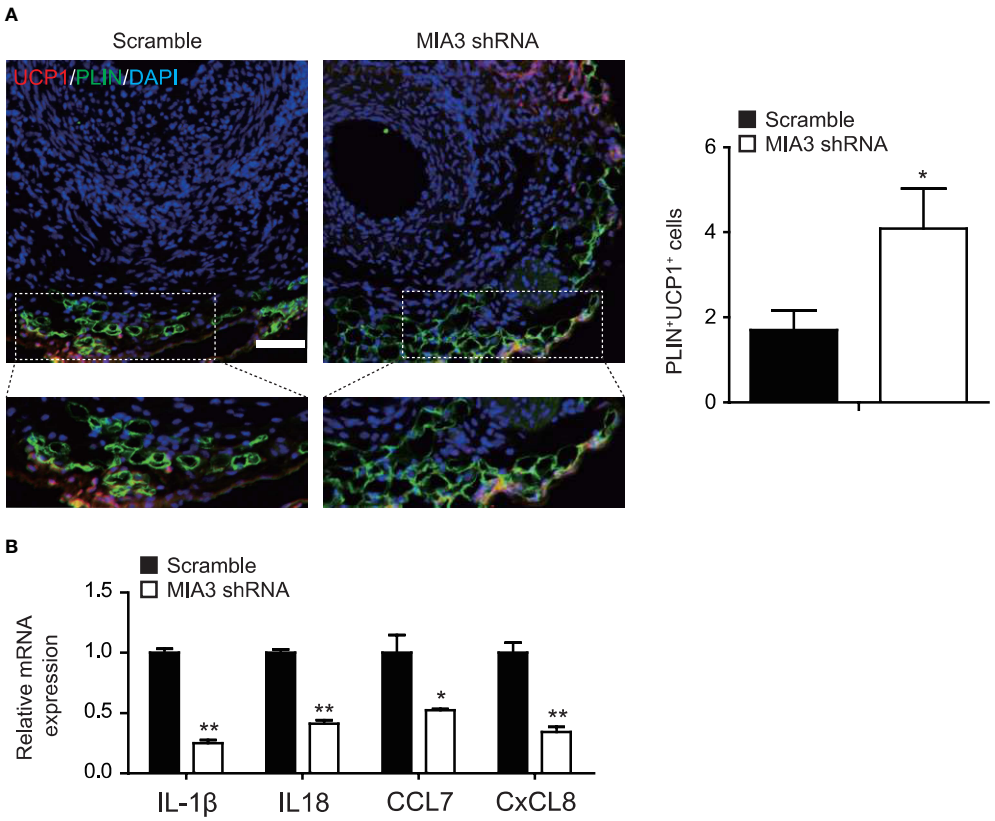

FIGURE 6 | Knockdown MIA3 increases the expression of uncoupling protein-1 in the perivascular adipocytes. **(A)** Knockdown MIA3 with shRNA lentivirus exhibited significant increase of PLIN and UCP-1 double-positive cells in the neointima at 28 days after femoral artery wire injury, with representative images (left) and quantification data (right; $n = 5$ for each group). Data are represented as the mean ± SEM. $^{*}p < 0.05$. *t*-test. **(B)** Knockdown MIA3 with siRNA in VSMCs significantly decreased expression of IL-1β, IL18, CCL7, and CxCL8 by quantitative real-time reserve transcription polymerase chain reaction. Data are represented as the mean ± SEM, $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$, *t*-test.

MIA3 might alleviate neointimal formation (24). We will pursue the role and mechanism of endothelial MIA3 in vascular injury neointimal formation in the future.

PVAT surrounds most large blood vessels and plays important roles in vascular homeostasis and excessive accumulation of dysfunctional PVAT leads to vascular disorders by targeting VSMCs and endothelial cells (10, 28). PVAT displays heterogeneity according to species and locations. Reversing the white features of PVAT to brown characteristics or maintaining PVAT beige features might be a crucial strategy to maintain a healthy vasculature (28). Our study revealed that MIA3 deficiency increased the expression of uncoupling protein-1 (UCP-1), the brown fat marker, in perivascular adipocytes, which indicated that MIA3 deficiency in VSMCs reversed the white features of PVAT to brown characteristics. RNA-sequencing bioinformatic analysis indicated 694 upregulated genes and 628 downregulated genes in MIA3-shRNA- and scramble-shRNA-treated VSMCs (Online uploaded excel file). The differentially expressed genes between scramble and MIA3 knockdown VSMCs were enriched for protein processing in endoplasmic reticulum, Hippo signaling pathway, TGF-β signaling pathway, etc. Knockdown MIA3 in VSMC decreased IL-1β, IL18, CCL7, and CxCL8 expression according to the RNA-sequencing bioinformatic analysis, which implies the important roles of inflammatory factors derived from VSMCs in injury-induced neointimal formation.

In summary, we present compelling evidence that MIA3 deficiency in VSMCs prevents neointimal formation by decreasing VSMC proliferation and migration and restoring BAT-like PVAT during injury-induced vascular remodeling. Our study found for the first time that inhibition of MIA3 in the injured arteries can prevent postangioplasty restenosis, supporting a potential role for MIA3 and its target genes in a variety of proliferative vascular diseases. These findings may have extensive implications for the treatment of occlusive vascular diseases.

DATA AVAILABILITY STATEMENT

The data presented in the study is deposited in the GEO repository, accession number GSE186951.

ETHICS STATEMENT

The animal procedures were performed in accordance with the Institutional Animal Care and Use of Laboratory Animals and were approved by the Animal Care Committee of Shanghai Jiao Tong University.

AUTHOR CONTRIBUTIONS

JZ conceived and designed the study. TZ organized and supervised the study. YL and JX conducted the experiments of this study. ML, TM, MC, YY, and HL conducted the statistical analysis. JZ and TZ drafted the article. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.748216/full#supplementary-material

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