Adipose tissue-derived extracellular vesicles: Systemic messengers in health and disease (Review)

XIAOBO YANG[1-4*], JIAYUE HAO[5*], JIE LUO[6*], XINLIANG LU[7] and XIANGHUI KONG[7]

[1]Department of Orthopedic Surgery, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310009; [2]Orthopedics Research Institute, Zhejiang University; [3]Key Laboratory of Motor System Disease Research and Precision Therapy of Zhejiang Province; [4]Clinical Research Center of Motor System Disease of Zhejiang Province, Hangzhou, Zheijiang 310002; [5]Institute of Immunology, Zhejiang University School of Medicine, Hangzhou, Zheijiang 310058; [6]Department of Gynecology, Women's Hospital, Zhejiang University School of Medicine, Hangzhou, Zheijiang 310006; [7]Bone Marrow Transplantation Center and Institute of Immunology of The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310058, P.R. China

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Abstract. Adipose tissue (AT) is a complicated metabolic organ consisting of a heterogeneous population of cells that exert wide-ranging effects on the regulation of systemic metabolism and in maintaining metabolic homeostasis. Various obesity-related complications are associated with the development of dysfunctional AT. As an essential transmitter of intercellular information, extracellular vesicles (EVs) have recently been recognized as crucial in regulating multiple physiological functions. AT-derived extracellular vesicles (ADEVs) have been shown to facilitate cellular communication both inside and between ATs and other peripheral organs. Here, the role of EVs released from ATs in the homeostasis of metabolic and cardiovascular diseases, cancer, and neurological disorders by delivering lipids, proteins, and nucleic acids between different cells is summarized. Furthermore, the differences in the sources of ADEVs, such as adipocytes, AT macrophages, AT-derived stem cells, and AT-derived mesenchymal stem cells, are also discussed. This review may provide valuable information for the potential application of ADEVs in metabolic syndrome, cardiovascular diseases, cancer, and neurological disorders.

Correspondence to: Dr Xinliang Lu or Dr Xianghui Kong, Bone Marrow Transplantation Center and Institute of Immunology of The First Affiliated Hospital, Zhejiang University School of Medicine, 866 Yuhangtang Road, Hangzhou, Zhejiang 310058, P.R. China
E-mail: xllu@zju.edu.cn
E-mail: 11818035@zju.edu.cn

*Contributed equally

Abbreviations: EVs, extracellular vesicles; ADEVs, adipose tissue-derived extracellular vesicles; T2D, type 2 diabetes; CVD, cardiovascular disease; AD, Alzheimer's disease; CH, cardiac hypertrophy; WATs, white adipose tissues; BATs, brown adipose tissues; BeATs, beige adipose tissues; MVs, microvesicles; ILVs, intraluminal vesicles; MVBs, multivesicular bodies; ncRNAs, noncoding RNAs; miRNAs, microRNAs; SCATs, subcutaneous ATs; DATs, dermal WATs; VAT, visceral ATs; ADSCs, adipose tissue-derived stem cells; ATMs, adipose tissue macrophages; IR, insulin resistance; GLUT4, glucose transporter 4; PPAR, peroxisome proliferator-activated receptor; FAs, fatty acids; FAO, fatty acid oxidation

Key words: Adipose tissues, Adipose tissue-derived extracellular vesicles, metabolic syndrome, Cardiovascular diseases, Cancers

Contents

1. Introduction

Obesity has rapidly become a widespread public health concern, the incidence of which has gradually increased over numerous decades. By 2025, the World Health Organization predicts that one in five adults will be obese globally (1). Obesity is a highly heterogeneous and complicated disorder caused by unbalanced energy metabolism. In addition, obesity is also closely associated with the pathogenesis of various metabolic diseases, such as type 2 diabetes mellitus (T2D), dyslipidemia, and cardiovascular diseases (CVDs), including hypertension and stroke, neurological disorders, musculoskeletal disease, and certain types of cancer (for example, breast, liver, ovarian, kidney, prostate and colon cancer) (2). Furthermore, several

studies have found that obese patients with associated comorbidities are more susceptible to SARS-CoV-2, exhibiting a higher risk of death (3-5).

Adipose tissues (ATs) are complex tissues that primarily exhibit a regulatory function. In mammals, ATs are primarily classified into white AT (WATs) and brown AT (BATs). In addition to metabolizing fat into energy, ATs also serve as a critical endocrine organ that can regulate energy metabolism, immunological responses, and cardiovascular balance by secreting a variety of adipokines, peptide hormones, and cytokines (6). Over the years, ~100 adipokines have been identified (7). Adipokines have a wide range of physiological effects on tissues and organs in different systems, such as the nervous system, immune system, and vascular system (8). For example, ATs secrete adiponectin that can promote insulin sensitivity and the antiatherosclerotic properties of cells by binding to adiponectin receptor (AdipoR) 1 and AdipoR2 (9-11). An inverse association exists between circulating adiponectin and obesity-related cancer incidence (11). Furthermore, adiponectin acts as an essential metabolic reprogramming factor by promoting the interaction between adaptor protein, phosphotyrosine interacting with PH domain, and leucine zipper 1 (APPL1) and AMP-activated protein kinase (AMPK), promoting glucose uptake through glucose transporter 4 (GLUT4) (12). The release of leptin by adipocytes was correlated with alterations in cell metabolism, such as the switch from mitochondrial β-oxidation to aerobic glycolysis (13). Chronic inflammation is another well-established characteristic of obesity (14). In obesity, there is an increase in oxidative stress and inflammation, leading to an increased release of proinflammatory adipokines, which can contribute to insulin resistance in the liver, muscles, and ATs, resulting in metabolic abnormalities (15). Studies have shown a positive correlation between obesity, insulin levels, insulin resistance, and increased tumor necrosis factor (TNF)-α production in human adipocytes (16).

In addition to the classical polypeptide adipokines and cytokines, ATs can also produce and secrete extracellular vesicles (EVs) (17). The composition of all EVs is similar to that of the parent cells, packed with bioactive molecules such as lipids, proteins, and DNA delivered to cells within ATs or in distant organs, mediating intercellular and interorgan communication (14). In this context, AT-derived extracellular vesicles (ADEVs) have been identified as crucial players in the cellular communication of immune and metabolic responses, regulating cellular processes in local and distant tissues (18,19). This review will focus on the compositions and functions of ADEVs from different cellular sources in ATs and their contribution to AT homeostasis and the development of metabolic complications, such as metabolic diseases, CVDs, several types of cancer, and neurological disorders.

2. Introduction to EVs

Initially, EVs were viewed as a quality control system to eliminate harmful or unnecessary molecules from the cell (20). EVs are now identified as a group of submicron-sized membrane-bound organelles secreted by almost all cells, carrying several biological cargoes, such as lipids, fatty acids (FAs), and nucleic acids, capable of targeting and transferring their contents to various receptor cells within the tissue or distal tissues (Fig. 1). EVs primarily consist of exosomes, microvesicles (MVs) and apoptotic bodies (21). Apoptotic bodies are known to be produced during apoptotic cell death and have a diameter >5 μm (22). MVs are small vesicles (100-1,000 nm size range) formed by plasma membrane fusion and budding. Although the exact process by which MVs are formed is not fully understood, cytoskeletal elements such as actin and microtubules, coat proteins, and fusion machinery, such as SNAREs, are hypothesized to be necessary. Specifically, coat proteins such as clathrin and cytoplasmic coat protein complex, are drawn to the membrane to reshape the flat membranes into rounded buds, cargo, and vesicle-SNAREs (v-SNAREs, primarily including VAMP) are integrated into the budding vesicle by attaching to coat subunits, for example, adaptor protein (AP) complexes (23). In addition, the molecular composition of MVs primarily consists of cytoplasmic and plasma membrane-associated proteins since MVs are formed by the outward budding of the membrane, and they may vary greatly depending on the cell type (24). In addition, MVs were first described as subcellular material originating from platelets and were demonstrated to play a role in blood coagulation (25,26). More recently, they have been reported to transfer cargo to target cells, thus playing an essential role in cell communication (27). Exosomes are vesicles 30-150 nm in diameter secreted by the endosome pathway. During the biogenesis of exosomes, endocytosis-mediated invagination of the plasma membrane (PM) forms early endosomes. Endosomal membranes bud inward into the lumen to create intraluminal vesicles (ILVs). These late endosomes contain ILVs called multivesicular bodies (MVBs). MVBs can fuse with lysosomes to be degraded or fuse with the PM to release ILVs as exosomes into the extracellular environment (28,29).

The nature and abundance of EV cargoes are specific to the cell type. They are frequently affected by the state of donor cells and the molecular processes that result in their biogenesis (30,31). EVs are loaded with various biomolecular components, such as nucleic acids and proteins, contributing to their functional diversity, heterogeneity, and complexity. Proteins commonly found in EVs are those associated with biogenetic mechanisms, including those related to endosomal pathways. Several membrane proteins and transcription factors can also be found in EVs (32,33). EVs are rich in sphingomyelin, cholesterol, desaturated lipids, phosphatidylserine, and ceramide (34). In addition, a range of genetic material is found in EVs, such as DNA, mRNAs, microRNAs (miRNAs), and several noncoding RNAs (ncRNAs). As soon as EVs bind to target cells, they may remain in the PM or be ingested through endocytosis, direct membrane fusion, and ligand binding mechanisms (35-37).

Proteins of the tetraspanin family, such as CD81 and CD9, are enriched in EVs and considered unique markers of EVs, including exosomes and MVs (38). However, researchers have demonstrated that CD81, CD63, and CD9 are exosome markers in a recent study on the difference between exosomes and MVs (39). At the same time, they emphasized that Annexin A1 is present in MVs, not exosomes. Furthermore, MVs also contain several biological molecules, such as integrins, selectins, and CD40 ligands, which may facilitate the formation of MVs (40,41). Therefore, more research is required to

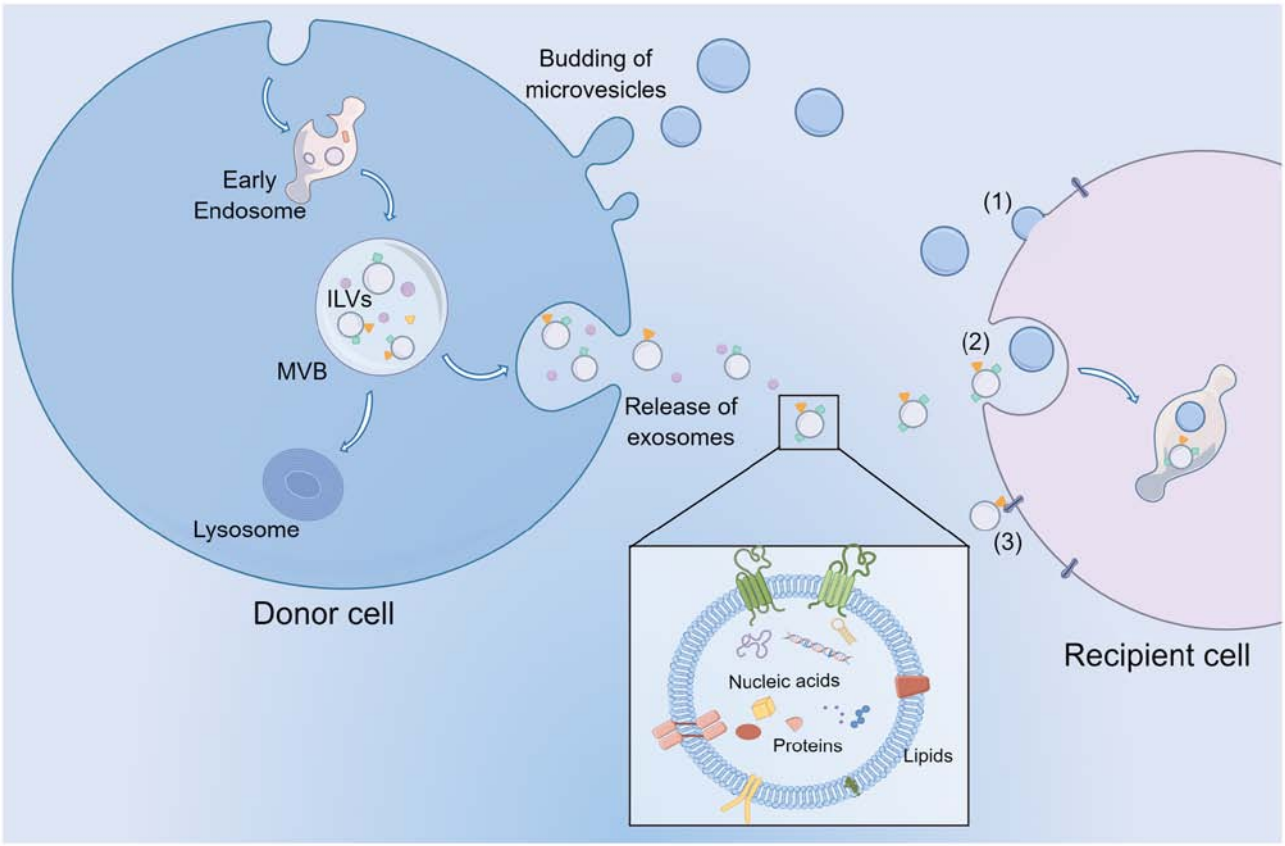

Figure 1. EV biogenesis, release, and communication with recipient cells. Exosomes and MVs released from cells are primarily stratified based on size. In addition, MVs can bud from the plasma membrane, while exosomes are derived from ILVs within the lumen of MVBs. MVBs then fuse with the plasma membrane to release the exosomes. EVs transport nucleic acids, proteins, and lipids to target cells by (1) membrane fusion, (2) endocytosis, and (3) ligand binding mechanisms to exert their biological functions. EV, extracellular vesicle; MVs, microvesicles; ILVs, intraluminal vesicles; MVBs, multivesicular bodies.

distinguish them from each other. In the present review, 'EVs' is used to refer to exosomes and MVs only.

3. AT and ADEVs

AT. ATs are complex metabolic organs with profound effects on regulating systemic metabolism, energy storage, and homeostasis. The primary characteristic of BATs is the presence of multilocular lipid droplets and several mitochondria expressing high levels of uncoupling protein 1 (UCP1), responsible for nonshivering thermogenesis, leading to increased energy expenditure (42). WATs primarily consist of white adipocytes, which carry large lipid droplets and fewer mitochondria, making them the primary site for storing and releasing energy (42). In addition, studies have shown that WATs can undergo a process called 'browning', during which part of the white adipose tissue can be transformed into beige adipose tissue (BeATs), morphologically distinct from WATs and BATs (43,44). Browning occurs under certain circumstances, such as in the cold and as a result of exercise. Moreover, medicines, such as β-adrenergic receptor and peroxisome proliferator-activated receptor (PPAR)γ agonists, can also trigger browning by promoting the decomposition of triglyceride and glucose in ATs or by inducing the expression of thermogenesis-related genes, respectively, which ultimately encourages lipolysis and thermogenesis (45-47). Characteristically, beige adipocytes contain several small lipid droplets, are typically larger than brown adipocytes, have more mitochondria than white adipocytes, and express UCP1 (48). Additionally, these types of ATs differ in critical ways that include aspects of their gene expression profile and secretome. WAT, known as the active endocrine organ, can release cytokines and adipokines such as leptin and adiponectin (6). In contrast, BAT/BeAT have fewer secretory functions than WAT. In addition to UCP1, Cell death-inducing DFFA like effector a, Cytochrome c oxidase subunit 7A1, and ELOVL fatty acid elongase 3 are specifically expressed in BAT, while BeAT expresses T-box transcription factor 1, Solute carrier family 27 member 1, Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1, CD40 and CD137 (49,50). Since the UCP1 expression of beige adipocytes is considerably lower than those of classical brown adipocytes, BeATs were once considered unimportant in whole-body energy expenditure (51). However, in adults with a minimal classic BAT reserve, BeATs are the primary energy source for nonshivering thermogenesis (52). Additionally, BeATs regulate whole-body energy metabolism and glucose homeostasis through an UCP1 independent mechanism (51). According to the report, activation of BeATs with β-adrenergic agonist CL316243 enhanced the selective uptake of fatty acids from triglyceride-rich lipoproteins by ATs, and reduced plasma TG and cholesterol levels, thereby alleviating hypercholesterolemia and atherosclerosis (53). Another study showed that prolonged maintenance of thermogenically active BeATs

enhanced whole-body energy expenditure and protected mice from diet-induced obesity and insulin resistance (54). As the most abundant form of AT, WAT is distributed throughout the body and are active endocrine organs. They release free fatty acids and adipokines, such as leptin, adiponectin, TNF-α, and IL-6, which act on distal tissues, including the brain, liver, and muscle tissue, to regulate food intake, energy homeostasis, and insulin sensitivity (55). The ATs found in the hypodermis layer are called subcutaneous ATs (SCATs), and form a connective tissue in the dermis between the aponeurosis and the muscle fascia, insulating and storing energy. SCATs are primarily distributed in the abdominal and gluteofemoral regions of the human body, storing >80% of total body fat (56). Dermal WATs (DATs), located directly below the reticular dermis (primarily above the SCATs), have been reported to be involved in insulation, hair regeneration, wound healing, and the prevention of skin infections (57,58). In addition, WATs that accumulate around internal organs are visceral ATs (VATs), which are primarily found in the intrathoracic region and abdominal cavity, such as epicardial and pericardial fat and perigonadal, mesenteric, perirenal, and retroperitoneal fat, protecting the internal organs of rodents and humans and storing 5-20% of total body fat (59,60).

Cellular composition of ATs. ATs are connective tissues primarily made up of lipid-rich cells known as adipocytes. Adipocytes, the parenchymal cells of ATs, are critical regulators of energy metabolism and endocrine modulators engaged in numerous physiological or pathological processes, such as appetite regulation and immunological response (61,62). In addition to adipocytes, there are several nonadipocyte compartments termed the stromal vascular fraction (SVF), composed of AT-derived stem cells (ADSCs), preadipocytes, endothelial cells, and a broad spectrum of adaptive and innate immune cells (63-66). Preadipocytes can differentiate into mature adipocytes to maintain adipogenesis and homeostasis in adipose tissue (67). The ADSCs in ATs are mesenchymal stem cells (MSCs) of mesodermal origin, serving as progenitors responsible for adipocyte regeneration and replenishment. ADSCs also have potent self-renewal capacity and a high capacity for classical adipogenic, osteogenic, and chondrogenic differentiation. In addition to mesenchymal cells, ADSCs can differentiate into nonmesenchymal cell lineages, such as endothelial cells, myocytes, and neuronal lineages (68). Endothelial cells and pericytes provide vasculature to ATs by forming capillaries (69-71). The immune cell types and functions of ATs have been widely discussed, primarily in the context of obesity. Various immune cells form a dynamic immunological microenvironment with variable metabolic status, including macrophages, eosinophils, dendritic cells (DCs), invariant natural killer cells (iNKT cells), T cells, and B cells (72-74). For example, under conditions of obesity or chronic metabolic stress, increased infiltration and activation of proinflammatory immune cells can accelerate WAT inflammation, thus influencing the effect of insulin and other metabolic hormones on parenchymal cells, thus further damaging the glucose and lipid metabolism process of metabolic organs (75-77).

Working model and the source of ADEVs. Although EVs are physiologically released from cells, pathophysiological stimuli can regulate their biogenesis and release. Furthermore, certain proteins and mRNAs can be selectively packaged into EVs during physiological changes or pathological injuries. Similar to normal EVs, ADEVs exert their biological functions by transporting bioactive cargos such as miRNAs, ncRNAs, proteins, and lipids to receptor cells. Studies have shown that ADEVs can not only modulate the immune responses of local ATs through cellular communication but can also regulate systemic insulin sensitivity and glycolipid metabolic processes through their remote effects on other metabolic organs (for example, the brain and liver) (78-80) (Fig. 2). Research has shown that ADEVs directly modulate glucose tolerance and insulin sensitivity in adipocytes, myocytes, and hepatocytes through modulation of PPARγ and perhaps fibroblast growth factor 21 (81,82). In the brain, ADEVs derived from adipocytes have been shown to carry the long noncoding RNA (lncRNA) metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) and to activate the mTOR signaling pathway through miR-181b and miR-144 in hypothalamic anorexigenic pro-opiomelanocortin (POMC) neurons, thereby leading to increased appetite and body weight (83). In addition, analysis of the protein profiles of EVs derived from adipocytes confirmed that EVs from obese mice are enriched in proteins and enzymes involved in the metabolism and transport of lipids, such as caveolin 1, lipoprotein lipase, and aquaporin 7, which may be associated with ectopic lipid accumulation and lead to mitochondrial energy metabolism disturbance, and systemic insulin resistance (84,85). In particular, quantitative proteomic analysis of EVs released by 3T3-L1 adipocytes showed that enzymes related to *de novo* lipogenesis, including glucose-6-phosphate dehydrogenase and fatty acid synthase, were selectively enriched in EVs from adipocytes, promoting lipid accumulation in recipient adipocytes and preadipocytes (85).

Most of the current body of knowledge on ADEVs comes from studies using 3T3-L1 cells. These studies found that ADEVs are highly adipocyte-specific and can be identified from complicated heterogeneous origins, such as plasma (86,87). Currently, multiple types of cells in ATs, such as adipocytes, ADSCs, and macrophages, have been shown to release EVs, mediating intercellular and interorgan crosstalk and regulating ATs and systemic homeostasis (88,89). EVs derived from adipocytes have been reported to carry proteins or enzymes involved in fatty acid oxidation (FAO), which can induce metabolic reprogramming and stimulate the migration and invasion of melanoma cells when EVs are taken up by tumor cells, thus amplifying the deleterious dialog between cancer cells and adipocytes (78,90). Furthermore, EVs derived from AT macrophages (ATMs) can modulate mouse glucose tolerance and insulin sensitivity (81,91). ADSCs have a high capacity for differentiation into multiple cell types and play an essential role in immune regulation (92-94). EVs released from ADSCs may at least be partially responsible for some of these functions. ADSC-derived EVs (ADSC-EVs) obtained from patients with or without cancer show equivalent miRNA content, which suggests that ADSC-EVs have the same therapeutic paracrine effects regardless of the health status of the donor (95). Previous studies have found that delivering ADSC-EVs from lean mice to obese mice showed desirable effects on alleviating obesity and IR (96). Additionally,

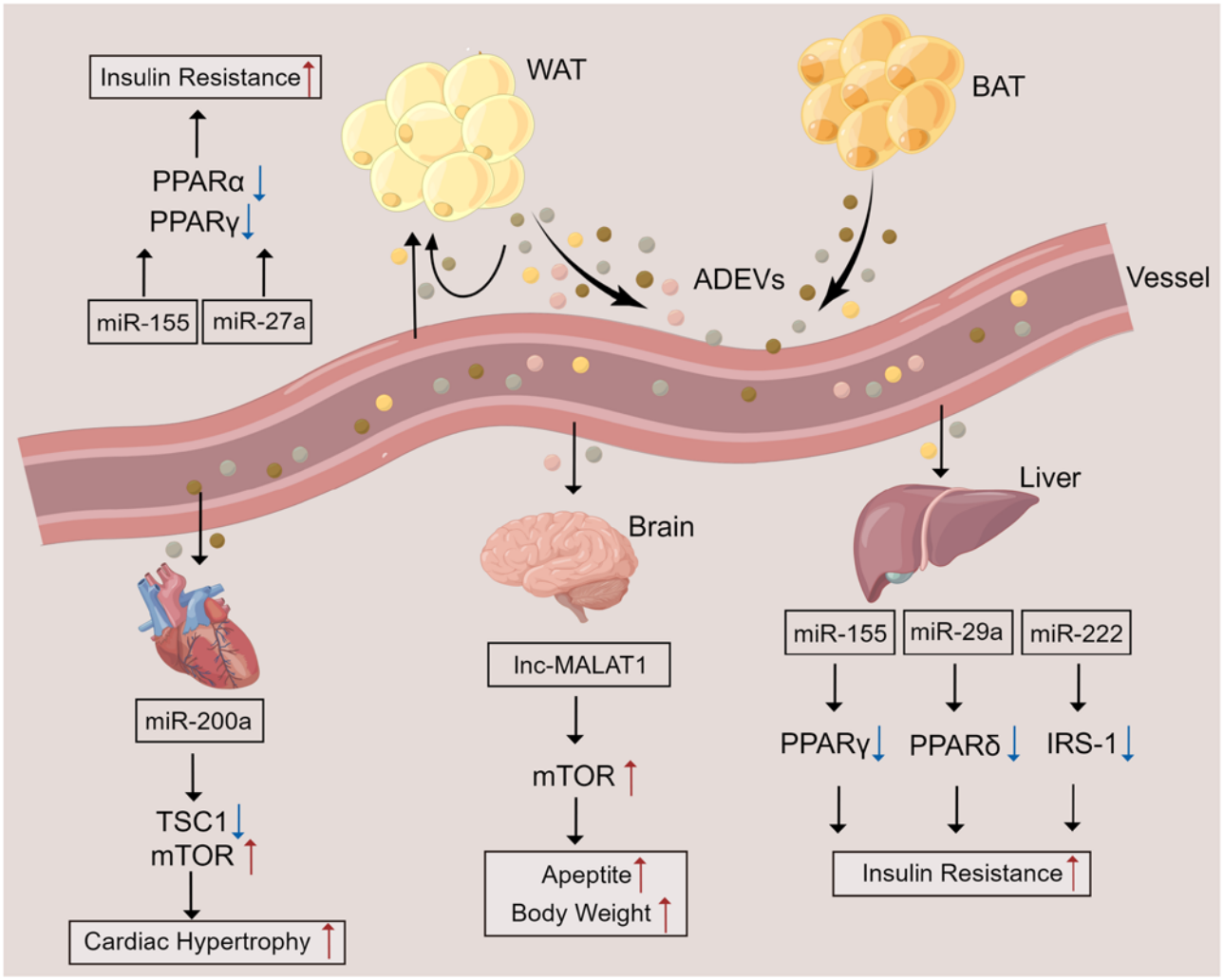

Figure 2. ADEV-mediated crosstalk between ATs and other organs. ATs secrete EVs containing various components into the blood circulation to affect ATs and distant organs. In ATs and the liver, miRNAs contained in ADEVs result in insulin resistance by inhibiting the activation or expression of PPARα, PPARγ, PPARδ, or IRS-1. In the brain, ADEVs contain lncRNA MALAT1, which enhances mTOR signaling in POMC neurons, leading to increased appetite and a gain in body gain. miR-200a contained in ADEVs can be delivered to cardiomyocytes to inhibit TSC1 and activate the mTOR pathway, leading to cardiac hypertrophy. ATs, adipose tissues; ADEVs, AT-derived extracellular vesicles; WAT, white AT; BAT, brown AT; miR, microRNA; lnc, long non-coding RNA; TSC1, tuberous sclerosis complex 1; PPAR, peroxisome proliferator-activated receptor; IRS-1, insulin receptor substrate 1; MALAT1, metastasis-associated lung adenocarcinoma transcript 1.

ADSC-EVs obtained from human WATs and BATs can induce ADSCs to differentiate into WATs and BATs, respectively, attenuating diet-induced obesity, glucose tolerance, and liver steatosis (97). These findings suggest that ADEVs can be used as cell-free therapeutics for AT regeneration and remodeling.

4. ADEVs in disease

ADEVs regulate metabolic disorders. AT dysfunction is accompanied by chronic low-grade inflammation, in which excessive adipokine synthesis and secretion are closely related to cardiovascular, chronic liver, and kidney diseases as well as other systemic metabolic disorders (98,99). The inflammation of WATs caused by obesity is characterized by the accumulation of macrophages, including monocyte-derived macrophages (moMacs). Moreover, obese WAT monocytes can differentiate locally into moMacs and contribute to ATM pools (100). During the development of obesity, macrophages transition from an anti-inflammatory phenotype to a proinflammatory phenotype, producing proinflammatory factors and exacerbating adipose inflammation (101). Initially, ADEVs were shown to be taken up by peripheral monocytes, which then differentiated into macrophages that increased TNF-α and IL-6 secretion, leading to insulin resistance and glucose intolerance through the Toll-like receptor 4/TIR domain-containing adaptor molecule 1 pathway (102). Notably, a recent study revealed a mechanism by which adipocytes regulate ATM polarization through EVs. ADEVs derived from mature adipocytes containing miR-34a inhibited macrophage M2 polarization by downregulating Krüppel-like factor 4, a crucial transcription factor for maintaining an adipose M2 macrophage phenotype, thereby leading to adipose inflammation (103). Several immunomodulatory proteins, such as macrophage migration inhibitory factor, retinol-binding protein 4, and soluble adiponectin, have been identified in EVs from cultured human adipocytes and human AT explants, which may induce monocytes to differentiate into M1-phenotype macrophages, exacerbating adipose inflammation and insulin resistance (86).

Insulin resistance (IR) is a disordered biological response in which the body cannot respond to high insulin levels or absorb and utilize glucose normally, causing the body to produce more insulin in response. It is the critical cause of several metabolic syndromes, such as T2D and obesity (104). As mentioned above, ADEVs from the VATs of obese mice were enriched in fatty acid binding protein 4 (FABP4) and induced the differentiation of macrophages to the M1 phenotype. In addition, those ADEVs were shown to be taken up by monocytes and to induce IR (102). Ying *et al* (91) found that injecting ATM-derived EVs from obese mice into lean mice inhibited the expression of PPARγ, which can promote whole-body lipid metabolism and insulin sensitivity, and GLUT4 (a PPARγ target gene), thereby reducing adipocyte sensitivity to insulin. Specifically, EVs containing miR-155 derived from ATMs target PPARγ in adipocytes, the liver, and the muscle, regulating insulin activity (91). In addition, adipocytes secrete EVs containing miR-27a, a negative regulator of PPARγ, leading to PPARγ-dependent obesity-induced IR by directly binding to the 3'UTR of PPARγ (105). Subsequently, ATM-derived EVs from obese mice were confirmed to possess miR-29a and to cause IR by binding to the 3'UTR of PPARδ (a potent modulator of insulin sensitivity) in adipocytes, myocytes, and hepatocytes (81). Another study showed that miR-222 from gonadal WAT-derived EVs promoted IR in the liver and skeletal muscle by suppressing IRS-1 expression via binding to the 3'UTR of IRS-1 (106). In contrast, ADSC-EVs facilitated metabolic homeostasis and improved insulin sensitivity in obese mice by alternatively activating M2 macrophage polarization and reducing inflammation by activating arginase-1 through STAT3 carried by EVs (96). In addition, miR-27a was enriched in adipocyte-derived EVs isolated from the VATs of obese individuals, and those EVs were shown to contribute to IR in the liver and skeletal muscle by inhibiting insulin-induced Akt phosphorylation and PPARα expression (107).

Given that obesity is a risk factor for the development of T2D, research has focused on the relationship between ATs and diabetes/diabetic complications. As no definitive marker of ADEVs has yet been identified, it is challenging to elucidate the detailed role of ADEVs in obesity and metabolic syndromes such as T2D. The production and specific cargo of ADEVs are altered under metabolic stresses (108). Perilipin A levels were higher in circulating adipocyte-derived EVs from obese mice and humans with metabolic diseases (109). AT-derived miRNAs are the main circulating EV miRNAs (18). A study showed that miR-20b-5p was abundant in serum EVs of T2D patients, and miR-20b-5p modulated insulin action in skeletal muscle by downregulating Akt interacting protein (AKTIP) and STAT3 expression (110). In addition, certain ADEVs exert beneficial effects on T2D. A previous study assessed the relationship between metabolic syndrome and adipose tissue-derived EV markers, and revealed that individuals with CD14-positive EVs had a 16% lower risk of developing T2D after 6.5 years of follow-up (111). EVs derived from activated beige adipocytes contain diabetes-preventing factors. When administered to primary white adipocytes, these EVs improved insulin sensitivity and insulin-stimulated glucose uptake (112). Together, based on these studies, adipose-derived EVs are viewed as a novel cellular communication tool within ATs and perhaps between ATs and distant organs to regulate T2D.

ADEVs and CVD. Dysfunctional ATs in obese individuals can lead to an increased risk of CVD, which remains one of the principal causes of death worldwide, despite advances in risk factor management (113-115). To date, efficient treatments for CVD are lacking. Recently, ADEVs have emerged as critical actors in the crosstalk between obesity and CVD progression.

Aside from polarization, macrophage foaming also plays a vital role in the progression of atherosclerotic lesions. ADEVs from VATs in obese mice facilitate macrophage foam cell generation by downregulating ATP binding cassette subfamily A member 1 (ABCA1) and ATP binding cassette subfamily G member 1 (ABCG1)-mediated cholesterol efflux and exacerbated atherosclerosis (116). ADEVs from adipocytes and their miRNA contents were confirmed to reduce macrophage cholesterol efflux by targeting ABCA1, thus promoting the development of atherosclerosis (117). In contrast, another study identified the beneficial effects of EVs derived from ADSCs in cardiac recovery, highlighting their potential in regenerative therapy (118). In addition, studies on 3T3-L1 models showed that adipocyte-derived EVs containing miR-802-5p promoted IR in cardiomyocytes by downregulating heat shock protein 60, which has been proven to prevent inflammation, mitochondrial dysfunction, and even insulin resistance (119).

Coronary artery disease and atherosclerosis are caused by endothelial dysfunction during the early stages of the disease (120). It is not well understood how EVs are exchanged between adipocytes and endothelial cells during obesity despite extensive evidence of proinflammatory crosstalk between ATMs and ADEVs. It was found that hypertrophic and dysfunctional adipocytes release EVs that impair vascular endothelial cell function, potentially contributing to obesity-related atherosclerosis (121). In addition, research has demonstrated that hypoxia and inflammation promote synergistic EV production from adipocytes. These ADEVs promote the expression of endothelial vascular cell adhesion molecule 1 (VCAM-1), which increases the subsequent attachment of leukocytes to endothelial cells and exacerbates vascular disease in obesity (122).

One of the causes of heart failure is cardiac hypertrophy (CH). Research has shown that miR-200a in ADEVs derived from adipocytes can be transferred into cardiomyocytes to inhibit TSC1 and activate the mTOR pathway, leading to CH. It was also shown that inhibition of miR-200a could abrogate CH (123). In addition, Gan *et al* (124) demonstrated that ADEVs derived from diabetic adipocytes were delivered to cardiomyocytes where they facilitated the pathogenic interaction between the heart and defective ATs, aggravating ischemic heart damage in obese/diabetic individuals. miR-130b-3p was found to be a vital agent mediating this proapoptotic effect of diabetic adipocyte-derived EVs and identified AMPK as a novel target of miR-130b-3p, in which miR-130b-3p was shown to impair the expression of AMPK, the latter of which is a crucial regulator of metabolic disorder-induced cellular malfunction and cell death (124). However, the precise role of ADEVs is still poorly understood in the context of CVD and requires further investigation.

ADEVs as major actors in cancer. An increasing body of data from animal and human studies indicates that obesity increases the risk of developing cancer, cancer-associated mortality, and cancer recurrence following treatment (125). Previously, studies on the communication between adipocytes and tumor cells have been limited to cytokines such as endorphin, leptin, or chemokines. Then, the discovery of cancer-associated adipocytes (CAAs) revealed a vicious cycle in which tumors activate CAAs, and CAAs can further contribute to tumor progression by secreting adipokines, inflammatory cytokines, and metabolites (126,127). More recently, the role of ADEVs in tumor–adipose tissue communication has also been confirmed, and obesity modifies ADEV secretion quantitatively and qualitatively, thus amplifying their effect on tumor aggressiveness.

The first line of evidence linking ADEVs with cancer showed that ADEVs from different adipocyte models enhanced melanoma cell migration, invasion, and lung metastases in the context of obesity (90). Subsequently, Wang *et al* (128) showed that EVs derived from 3T3-L1 adipocytes induced lung tumor metastasis by increasing MMP9 activity of 3LL lung cancer cells, in which MMP9 has been shown to promote tumor invasion and metastasis. Another study demonstrated that ADEVs from AT-derived mesenchymal stem cells (ADMSCs) could foster the invasion, migration, and proliferation of osteosarcoma cells by increasing galactosyl transferase 2 and MMP2/9 expression (129). Gangadaran *et al* (130) demonstrated that ADSC-EVs contain angiogenic proteins such as IL-8, CCL2, TIMP-1, TIMP-2, and VEGF-D. Following internalization of ADSC-EVs, endothelial cells undergo differentiation, develop a tube-like formation and promote angiogenesis *in vitro* and *in vivo*. Khanh *et al* (131) showed that in patients with T2D, ADEVs derived from ADMSCs can promote breast cancer metastasis by targeting the JAK/STAT3 pathway. In conditions of obesity, ADEVs from AT macrophages are rich in miR-155, which is not only involved in IR but also plays an oncogenic/antiapoptotic role through caspase-3 and Bcl-2 in breast cancer cells (132).

By contributing local FAs to the process of FAO within tumor cells, a novel beneficial metabolic route is activated that increases tumor aggressiveness and proliferation, and adipocytes also aid in the evolution of tumors through metabolic collaboration (90,133,134). The proteins, including the enzymes needed for FAO, can be transferred to cancer cells or across farther distances by ADEVs released by adipocytes. Lazar *et al* (90) demonstrated that proteins implicated in FAO were enriched in ADEVs, which were then taken up by melanoma cells, leading to increased lung metastases and an increase in FAO in tumor cells. Clement *et al* (78) revealed the role of ADEVs in the crosstalk between melanoma cells and adipocytes, which triggers metabolic remodeling and ultimately facilitates the FAO process and tumor aggressiveness. In addition to FAO enzyme transfer, ADEVs also deliver FAs to tumor cells to enhance the FAO process, reinforcing the effect of ADEVs on obesity. Together, this research revealed that ADEVs are involved in guiding the growth, invasion, metabolic reprogramming, and metastasis of cancer cells by modulating the acquisition and maintenance of cancer markers.

Effects of ADEVs on neurological disorders. Neurological disorders, including neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD), as well as ischemic stroke, are often characterized by neuroinflammation. Treating neurological disorders has long been challenging as many therapeutics do not cross the blood-brain barrier (BBB). To date, EVs carrying biological molecules are recognized as an excellent tool for treating central nervous system (CNS)-related ailments since they are prospective drug delivery systems that can cross the BBB (135).

The deposition of β-amyloid peptide (Aβ) in the brain and neurofibrillary tangles (NFTs) formed by hyperphosphorylated Tau protein plays a critical role in the pathogenesis of AD (136,137). According to Katsuda *et al* (138), ADSC-derived EVs carried enzymatically active neprilysin (NEP), the brain's most important Aβ-degrading enzyme. Furthermore, Aβ levels were decreased when ADSC-derived EVs were transfused into N2a neuroblastoma cells *in vitro*. Another study demonstrated that ADSC-derived EVs inhibited inflammatory polarization of activated human microglia, which has been shown to mediate several CNS inflammatory processes, such as AD (139). In another rat model of ischemic stroke, ADSC-derived EVs containing miR-126 were shown to prevent ischemic stroke, promote neurogenesis, and vasculogenesis following ischemic stroke, and inhibit ischemic stroke-induced microglial activation and inflammatory responses (140). Jiang *et al* (141) showed that EVs derived from ADSCs suppress autophagy by inhibiting the expression of Beclin-1 and Atg5 via miR-30d-5p, thereby promoting microglial M2 polarization and ultimately preventing acute ischemic stroke. These characteristics make ADSC-derived EVs promising candidates for therapeutic relevance in neurodegenerative diseases.

5. Conclusions and future directions

In addition to the classical polypeptide adipokines and cytokines, the critical role of ADEVs in communication between ATs and other organs has been gradually deciphered. More recently, growing evidence from both epidemiologic and preclinical studies further highlights the effects of ADEVs on mediating cell-to-cell communication within ATs, and between ATs and other peripheral organs. A comprehensive understanding of the interaction patterns between ATs and other tissues and the molecular changes in AT dysfunction during obesity, such as ADEVs and their cargoes, may provide novel avenues for developing new therapeutic interventions in obesity-related metabolic diseases.

EVs have long been recognized as crucial intercellular communication tools. In addition, EV-mediated cellular communication is implicated in various diseases and biological events, including certain immune responses such as inflammation. Therefore, EVs are also considered a therapeutic target for multiple diseases (142). In addition, due to their endogenous origin, EVs have been widely explored as next-generation nanoscale drug delivery systems, allowing them to circumvent certain drawbacks associated with existing therapies (143). The impact of obesity on the biological components of ADEVs from different cell origins has been described previously (144). Therefore, the critical function of ADEVs and the role of ADEVs and their cargoes in multiple diseases were emphasized here.

Table I. Summary of ADEV cargos and their functions in recipient cells.

A, ADEVs in metabolic disorders

Origin	Cargo	Functions	(Refs.)
Adipose tissue-EVs	N.D.	Promote M1 polarization of macrophages; Induce IR	(102)
Adipocyte-EVs	miR-34a	Inhibit M2 polarization of macrophages	(103)
Human adipocyte-EVs	MIF, M-CSF, TNF-α	Promote M1 polarization of macrophages	(86)
ATM-EVs	miR-155	Reduce the insulin sensitivity in adipocytes, the liver, and the muscle	(91)
Adipocyte-EVs	miR-27a	Induce hepatic and skeletal muscle IR	(105,107)
ATM-EVs	miR-29a	Induce IR in adipocytes, myocytes, and hepatocytes	(81)
WAT-EVs	miR-222	Promote IR in the liver and skeletal muscle	(106)
Human adipocyte-EVs	miR-20b-5p	Impair insulin action in skeletal muscle	(110)

B, ADEVs and CVDs

Origin	Cargo	Functions	(Refs.)
VAT-EVs	N.D.	Facilitate macrophage foam cell generation and exacerbate atherosclerosis	(116)
Adipocyte-EVs	miRNAs	Increase macrophage cholesterol efflux	(117)
3T3-L1-EVs	miR-802-5p	Promote insulin resistance in cardiomyocytes	(119)
Adipocyte-EVs	N.D.	Impair the function of vascular endothelial cells	(121)
Adipocyte-EVs	N.D.	Promote the attachment of leukocytes to endothelial cells	(122)
Adipocyte-EVs	miR-200a	Impair the function of cardiomyocytes, and promote the process of cardiac hypertrophy	(123)
Adipocyte-EVs	miR-130b-3p	Exacerbate ischemic heart injury	(124)

C, ADEVs in cancer

Origin	Cargo	Functions	(Refs.)
Adipocyte-EVs	MMP3	Promote lung tumor metastasis	(128)
ADMSC-EVs	N.D.	Promote the invasion, migration, and proliferation of osteosarcoma cells	(129)
ADSC-EVs	Angiogenic proteins	Promote the tube-like formation of endothelial cells	(130)
ADMSC-EVs	N.D.	Promote the metastasis of breast cancer cells	(131)
Adipocyte-EVs	FA substrates, proteins implicated in FAO	Promote lung tumor metastasis	(90)
Adipocyte-EVs	FAO enzyme, FA substrates	Trigger metabolic remodeling, facilitate FAO and tumor aggressiveness	(78)

N.D., not detected; ADEVs, adipose tissue-derived extracellular vesicles; ATM, adipose tissue macrophage; WAT, white adipose tissue; VAT, visceral adipose tissue; ADSC, adipose tissue-derived stem cells; ADMSC, adipose tissue-derived mesenchymal stem cells; FAO, fatty acid oxidation; FA, fatty acids; miR/miRNA, microRNA; IR, insulin resistance; MIF, macrophage migration inhibitory factor; M-CSF, macrophage colony stimulating factor 1; TNF-α, tumor necrosis factor-α; MMP3, matrix metallopeptidase 3.

The present review summarizes the composition and function of ADEVs derived from different cell sources in ATs, such as adipocytes, preadipocytes, macrophages, and MSCs. In addition, ADEVs participate in developing pathologies associated with metabolic diseases, CVDs, and several types of cancer (Table I). Understanding the mechanisms behind the effects of ADEVs on obesity or metabolic disorders and CVDs may contribute to the development of novel therapeutic strategies. However, the vast majority of current research is currently in the early stages, and no definitive marker of ADEV has yet been identified, complicating the isolation of ADEVs from ATs with high purity. In current research models, ADEVs from different sources are frequently derived from *in vitro* cell cultures. Therefore,

further investigation is required to reveal the detailed characteristics of ADEVs.

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Authors' contributions

XBY, JYH, and JL wrote the manuscript. XHK and XLL conceived the subject of review and edited the manuscript. XHK designed and created the schematic representations. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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