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Intercellular and interorgan crosstalk through adipocyte extracellular vesicles

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Abstract

Functional adipose tissue is essential for homeostatic maintenance of systemic metabolism. As such, adipose tissue dysfunction, like that seen in the obese state, directly contributes to system-wide pathological metabolism, leading to the development of type 2 diabetes and other obesity-associated comorbidities. In addition to the storage function of adipocytes, they also secrete numerous factors that robustly regulate metabolism-related pathways throughout the body. Many of these factors, in addition to other signaling proteins, RNA species and lipids, are found in extracellular vesicles (EVs) released from adipocytes. EVs are vesicles with a lipid bilayer, known to carry signaling proteins and lipids, mRNAs and miRNAs. Because of this diverse cargo, EVs can have robust and pleotropic signaling effects depending on the receiving target cells. We are only now starting to understand how adipocyte EVs can modulate metabolism within adipose tissue and beyond. Here, we highlight the current literature that demonstrates EV-mediated crosstalk between adipocytes and other tissues or distal cells. We become increasingly aware of the importance of these adipocyte-derived EV signals that establish a so far underappreciated endocrine system. Adipocyte EVs offer a new avenue for pharmacological manipulation of metabolism to treat obesity-related disease.

Keywords Exosome · Extracellular vesicle · Adipose tissue · Adipocyte · Obesity · Diabetes

Abbreviations

EV	Extracellular vesicle
sEV	small extracellular vesicle
lEV	large extracellular vesicle
AT	adipose tissue
Ad	adipocyte
MVB	multivesicular body
CAAs	cancer-associated adipocytes
hMSCs	human mesenchymal stem cells
ECM	extracellular matrix
HASCs	adipose-derived stem cells
ECs	endothelial cells

White adipose tissue (AT) serves essential functions that regulate systemic metabolism during periods of starvation or nutrient abundance. The dynamic adaptation of adipose tissue to these divergent nutrient stresses is indispensable for health and survival. This is, in part, due to the ability of adipocytes to store excess calories in the form of lipids, a function that provides a mobilizable source of energy and protects other organ systems from lipotoxicity. Beyond storage, adipocytes secrete an impressive array of macromolecules that include classic adipokines, such as adiponectin and leptin, as well as lipids, RNA species and metabolites [1]. Together, the signaling capabilities of these molecules are extensive, and include modulation of inflammation, insulin sensitivity, weight gain, reproduction, energy expenditure and many additional functions related to interorgan communication [1]. Consequently, the adipocyte secretome can regulate cellular functions in an autocrine, paracrine and endocrine way. The impact of adipocyte-derived signals has become evident in human and mouse studies of obesity where dysfunctional adipocytes lead to AT hypoxia, fibrosis and inflammation, all of which contribute to whole body metabolic disturbances. In fact, dysfunctional adipose tissue promotes system-wide pathological processes, such as type 2 diabetes, cancer, cardiovascular and neurological diseases as well as immune dysfunction [2].

Recent studies have shown that much of the human AT protein secretome and the majority of the miRNA secretome can be found in adipocyte-derived extracellular vesicles (EVs;

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[3, 4]). EVs are a heterogenous population of membrane enclosed nano-particles released from most cells, with the adipocyte suggested to represent one of the most prominent sources of EVs in the circulation. They carry signaling proteins, lipids, mRNA and miRNAs that induce coordinated activation of signaling pathways in recipient cells [5]. As such, EV-mediated signaling can integrate the functional activation or suppression of multiple pathways simultaneously at all levels of regulation: mRNA, protein, and post translational modifications [4–6]. This offers a mode of signaling that is more powerful than soluble secreted hormonal factors alone. In addition to intercellular signaling, EVs have also been proposed as a mechanism to remove damaged, toxic or unneeded cellular components [7, 8]. These differences in EV function are likely determined by their intracellular origin. EVs are broadly characterized into two types, exosomes and microvesicles. Exosomes are small EVs (50-150 nm) that are formed by the inward budding of the endosomal membrane in the multivesicular body (MVB; [5]). Fusion of the MVB with the plasma membrane results in the release of exosomes into the extracellular space. In contrast, microvesicles tend to be larger EVs (50–1000 nm) and bud directly from the plasma membrane [5]. Both types of EVs contain proteins and nucleic acids that are differentially enriched and have robust signaling capacity [5]. Because of the biochemical similarities, current isolation techniques only allow for a distinction between small EVs (sEV; an exosome-enriched population that also contains small microvesicles) and large EVs (lEVs; microvesicles >200 nm). Our knowledge of EV biogenesis, cargo sorting, cell targeting mechanisms and the biological significance of EV heterogeneity are limited [9]; however, EVs have been shown to modulate a large number of cellular processes and disease states ranging from cancer to mental disorders [10, 11]. The insights into EV-mediated metabolic regulation are rapidly advancing, and adipocyte-derived EVs seem to contribute systemically to a significant extent.

1 Molecular features of adipocyte EVs

Multiple studies have contributed to a large body of data that characterizes adipocyte-specific or AT EV cargo with the use of proteomics, lipidomics and microarrays [3, 4, 12–18]. This has provided us with functional insights, which will be discussed in detail below. However, one must be careful with the interpretation of these studies, as many assess AT EVs and not EVs from *in vitro* cultured adipocytes. Although there is evidence that adipocytes contribute the majority of EVs to the adipose tissue population [3, 16, 17], sEVs derived from other adipose tissue resident cells can also have strong signaling effects. For example, adipose tissue macrophages from obese mice release sEVs that are sufficient to cause systemic insulin resistance when injected into lean mice [19]. Nevertheless, the accumulated omics data is indispensable for our functional understanding of adipocyte sEVs as well as guidance for how we can distinguish adipocyte sEVs from other cell-derived EVs in a mixed population like that of serum or plasma.

The proportion of circulating sEVs that are adipocyte-derived has not been definitively quantified. Studies done on adipocyte-specific dicer KO mice showed that the majority of circulating sEV-associated miRNA are of adipocyte origin, suggesting adipocytes are the dominant source of blood sEVs [4]. However, others claim, using an adipocyte-specific genetic tag, that adipocyte sEVs make less of a contribution to the circulating EV pool [17]. Thus, new approaches are needed to demonstrate the relative abundance of adipocyte- vs. other cell-derived sEVs in circulation. There is merit in utilizing the endogenously incorporated adipocyte macromolecules to identify adipocyte sEVs, which can be semi-quantitively compared across experimental conditions. Adipocyte-specific EVs in biological fluids can be identified using three characteristics: adipocyte marker proteins, adipocyte-enriched miRNAs and the presence of triglycerides and fatty acids (Fig. 1). Adipocyte EVs carry the adipokines adiponectin and resistin but not leptin [8, 15, 20–23]. However, only a trace amount of resistin, and about 4% of total circulating adiponectin are detected in EVs [22, 23]. Therefore, the presence of these adipokines may be helpful markers to indicate adipocyte origin but, based on abundance, may not be functionally important. That said, the physiological

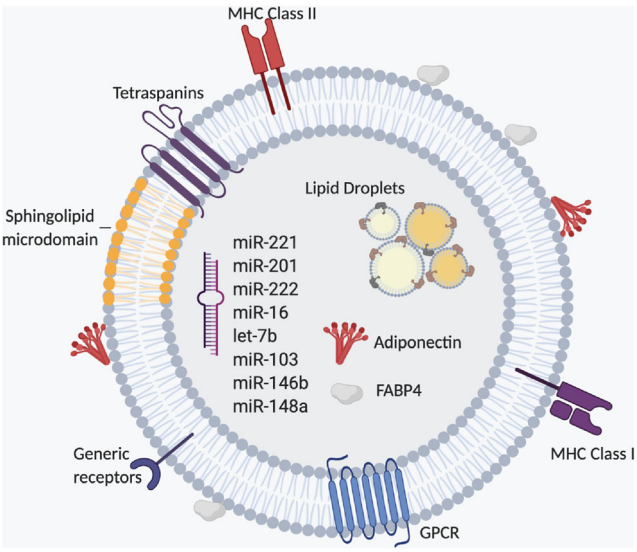

Fig. 1 Ad-EVs contain a unique signature of macromolecules: lipid droplets, adipocyte-enriched miRNAs, FABP4, and adipokines like adiponectin. Ad-EVs also contain other protein and lipid cargo that are a characteristic of all EVs regardless of the producing cell type: cell membrane proteins such as tetraspanins and MHC class I and II molecules, cell surface receptors such as GPCRs and other generic receptors, and sphingolipid-rich membranes

relevance of EV-associated adipokines should not be disregarded completely as resistin in EVs has been implicated in the development of high-fat diet induced liver steatosis [20]. Other than adipokines, FABP4 is highly enriched in adipocyte EVs and has been used to qualitatively assess adipocyte-specific EVs in circulation [15, 24, 25]. Interestingly, a category of proteins that are identified in most proteomics analysis of adipose tissue or adipocyte EVs corresponds to mitochondrial resident proteins [3, 13, 15, 26]. In fact, a study by Lazar et al. demonstrated that adipocytes were the only cell type of the ones tested to release mitochondrial fatty acid oxidation enzymes in sEVs [12]. This suggests mitochondrial proteins in circulation may constitute an adipocyte-specific EV signature. This, however, needs to be further demonstrated and the *in vivo* relevance further studied. Beyond proteins, several miRNAs may also be good indicators of adipocyte-derived sEVs. miR-221, miR-201, miR-222 and miR-16, let-7b, miR-103, miR-146b, and miR-148a incorporate into sEVs, all of which are miRNAs that are highly enriched in adipocytes [4, 27]. Finally, the presence of triglycerides and fatty acids have recently be shown to indicate at least a subpopulation of sEVs with adipocyte origin [17, 26, 28]. However, only sEV isolation by size exclusion chromatography or ultrafiltration will yield neutral lipid-containing vesicles, as this population of EVs floats during ultracentrifugation [17]. Thus, these molecular features of adipocyte sEVs can be leveraged in both mice and humans to assess changes in this cell-specific population across disease states.

2 Regulation of adipocyte EV release

The production and release of the sEV subpopulation called exosomes is associated with conditions of cellular stress. In cultured adipocytes, enhanced sEV release has been reported in response to hypoxia, pro-inflammatory cytokines, and fatty acids [14, 15, 22, 29, 30]. The molecular mechanisms that trigger sEV release in response to stress have not been elucidated, although palmitate-stimulated sEV production likely occurs through ceramide generation. Increases in intracellular ceramides are well-known stimulators of exosome release from many cells [31]. As palmitate drives *de novo* ceramide production, it too triggers exosome release. Other sources of stress, such as hypoxia and inflammation, have not yet been linked with ceramide-induced sEV production. Although it is of interest that that sEV release is stress stimulated, further work is required to determine the physiological significance of this event. Is the primary physiology of sEVs produced under stress to signal to other tissues? Or is the cell attempting to extrude damaged or toxic cellular components that build up during stress? In this case the resulting signaling events elicited in other tissues are secondary. It is likely that stress-stimulated sEVs serve both functions.

3 Adipocyte EV-mediated crosstalk with macrophages

Adipocyte-derived EVs (Ad-EVs) have been shown to modulate the function of multiple AT resident or infiltrating cells including macrophages, cancer cells, endothelial cells, adipocyte progenitors and mature adipocytes (Fig. 2). Although our understanding of these tissue EV-mediated signaling events is limited, the communication axis that is the most well-studied is that between adipocytes and macrophages. This interaction is of great interest in obesity and type 2 diabetes research, as AT macrophage proinflammatory activation participates in the pathology of metabolic syndrome [32]. Many studies have reported this sEV-mediated crosstalk by demonstrating the transfer of adipocyte-specific mRNAs and proteins or adipocyte-expressed membrane exogenous tags to macrophages [17, 18, 27, 33]. Early studies demonstrated that this inter-cellular transfer of EVs is functionally important. sEVs isolated from the AT of genetically obese *ob/ob* mice are taken up by circulating monocytes, triggering differentiation into pro-inflammatory macrophages [34]. In turn, the activated macrophages induced insulin resistance in C2C12 myoblasts [34]. Through this mechanism, injections with sEVs from obese AT were sufficient to cause whole body glucose intolerance and insulin resistance [34]. Although the sEVs used in this study were not adipocyte-specific, others have demonstrated a similar effect using sEVs from cultured adipocytes. In these studies, sEVs were isolated from mature primary, floated adipocytes from obese mice or 3 T3-L1 *in vitro*-differentiated adipocytes treated to mimic the obese state (cultured under high glucose and insulin conditions). Both adipocyte small EVs (Ad-sEVs) and adipocyte large EVs (Ad-lEVs) can induce migration and differentiation of monocytes into an M1-like proinflammatory state *in vitro* and *in vivo* [17, 24, 35–37]. These effects have been proposed to occur through multiple mechanisms, including Sonic Hedgehog (Shh), RBP4 and miR-155, all reported cargo of Ad-EVs [30, 34–36]. Interestingly, Ad-sEVs actively suppress the polarization of macrophages to the anti-inflammatory M2-like state via miR-34a targeting of Kruppel-like factor 4 Klf4 [38]. In addition to macrophage activation, Ad-EVs also promote lipid accumulation in macrophages by direct transfer of lipid droplets [17]. The catabolism of these lipids in the macrophage is associated with inflammation-mediated insulin resistance [39]. This EV-based communication is also reciprocated from the macrophage to the adipocyte, although very few studies provide further insights into this process. sEVs from LPS-stimulated macrophages propagate a pro-inflammatory gene signature in the receiving adipocytes [40].

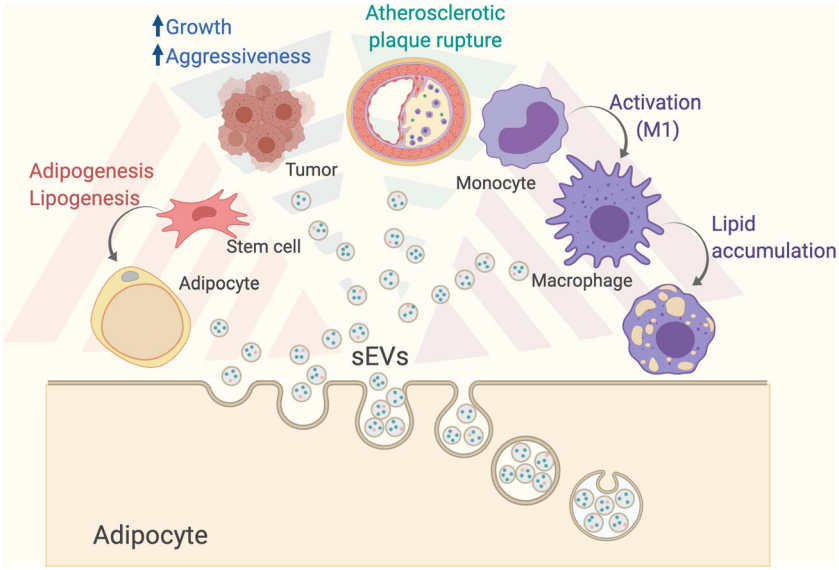

Fig. 2 Adipocyte-specific, or AT EVs signal locally to enhance differentiation and lipid accumulation in pre-adipocytes, promote increased growth and aggressive of cancer cells, induce pathological angiogenesis and destabilize atherosclerotic plaques, and induce differentiation of monocytes into pro-inflammatory, lipid laden macrophages

4 Adipocyte EV-mediated crosstalk with cancer cells

Another well-established EV signaling axis is between adipocytes and cancer cells. Obesity is associated with a higher risk of carcinogenesis in the breast, uterus, prostate, stomach, kidney, gall bladder, esophagus, colon and liver [41]. Furthermore, obesity is linked to faster progression of cancer and increased cancer-associated mortality [41]. Many systemic factors have been implicated in this association, such has hyperinsulinemia, hyperglycemia, hyperlipidemia, and inflammation, all of which are consequences of dysfunctional AT in obesity [42]. Cancer cells in close proximity to adipocytes, such as seen in breast cancer lesions, establish a metabolic symbiosis with surrounding adipocytes that are also referred to as cancer-associated adipocytes (CAAs). Cancer cells reprogram CAA metabolism at the invasive front so that they resemble a beige/brown adipocyte phenotype, therefore being highly catabolic and energy-dissipating. These browned adipocytes release metabolites such as lipids, ketone bodies, and amino acids to meet the high metabolic demands of the proliferating cancer cell [43–45]. Consequently, CAAs take on a delipidated morphology [46]. This lipolytic and browning phenotype is thought to promote cancer associated cachexia, the wasting of skeletal muscle and unintentional loss of AT [47, 48]. In addition, CAAs secrete altered ratios of adipokines, cytokines, inflammatory factors and growth factors to further fuel tumor growth [41]. In recent years, it has become increasingly clear that EVs relay CAA-cancer cell signals in the tumor microenvironment and between organs. Cancer cell-derived sEVs can target proximal adipocytes or distal adipocytes to promote lipolysis and a brown-like adipocyte phenotype. For example, pancreatic cancer cells release sEVs into circulation that induce lipolysis in subcutaneous AT via sEV adrenomedullin activation of p38 and ERK1/2 MAPKs [49]. A similar phenotype is reported in breast cancer models where sEV-associated miR-155 and miR-144 from breast cancer cells down-regulated PPARγ expression, which in turn resulted in beiging of white adipocytes [44, 50]. This was also the result of p38 activation and ERK1/2 suppression [44, 50]. sEV miR-126 from breast cancer cells has been shown to suppress glucose metabolism in adipocytes by disruption of IRS/GLUT4 signaling, activation of AMPK and stabilization of HIFα [44]. Another study demonstrated that sEVs from Lewis lung carcinoma (LLC) cells stimulate lipolysis in 3 T3-L1 adipocytes, and white adipocytes *in vivo* [48]. Pharmacological reduction of sEV production in LLC tumor-bearing mice attenuated cancer-associated cachexia [48]. Lastly, sEVs from hepatocellular carcinoma cells induce a pro-inflammatory phenotype in adipocytes [51]. Therefore, EVs from various types of cancer cells can rewire adipocyte metabolism and function to promote tumor growth.

Adipocytes also respond to cancer cell signals by releasing EVs. In the context of invasive melanoma, where cancer cells are in direct contact with dermal adipocytes, adipocyte-derived sEVs stimulate an aggressive cancer phenotype [12]. Mechanistically, Ad-sEVs contain fatty acids and mitochondrial fatty acid oxidation enzymes, which enhance lipid oxidation in melanoma cells [12, 26]. Ad-sEVs can also transfer miR-23a/b or circRNAs that promote the growth of hepatocellular carcinomas by targeting the VHL/HIF axis or deubiquitination-related USP7 respectively [52, 53]. In all cases, the adipocyte-cancer cell interactions through EVs is

advantageous for tumor growth, migration, survival and thus, enhances the aggressiveness of disease progression.

5 Adipocyte EV signaling to other AT cells

We have some evidence that preadipocytes, endothelial cells and mature adipocytes are targets of Ad-EVs, although much less is understood about these Ad-EV communication lines compared to that of macrophages and cancer cells. Studies have claimed that cultured adipocytes, either healthy, hypoxic, insulin resistant or hypertrophic, release sEVs that can promote the differentiation of preadipocytes [14, 24, 54]. However, these studies did not distinguish between EV-mediated enhancement of differentiation and increased lipid accumulation through lipogenesis. Particularly in the case of hypoxia where adipocyte EVs transfer enzymes in the *de novo* lipogenesis pathway to 3 T3-L1 preadipocytes, suggesting a lipogenic, not differentiation effect [14]. That said, there is some evidence that Ad-sEVs can determine the lineage of human mesenchymal stem cells (hMSCs; [55]). As hMSCs can differentiate into multiple cell types in the mesodermal lineage, their fate is directed by multiples cues, including secreted factors or mature cell extracellular matrix (ECM) constituents. Ad-sEVs work synergistically with adipocyte ECM to direct hMSCs into the adipocyte lineage. Interesting, Ad-sEVs can override the ECM signals so that Ad-sEVs stimulate hMSCs differentiate into adipocytes even when grown on osteogenic ECM [55]. Another study demonstrated sEVs isolated from the conditioned media of human adipose-derived stem cells (HASCs) during differentiation into either beige or white adipocytes are sufficient to induce differentiation of naïve HASCs to the respective beige or white lineage [56]. Injection of high fat-fed mice with sEVs from beige-differentiating HASCs resulted in attenuation of diet-induced whitening of brown adipose tissue, reduced weight gain and amelioration of liver steatosis [56]. Therefore, sEVs from both mature adipocytes and those early in differentiation can influence the lineage of surrounding precursor populations.

Adipocytes can also use EVs to communicate with other adipocytes in the tissue. Although less studied, this EV-mediated exchange of cellular components between adipocytes seems to serve as a way to propagate adaptive responses, to synchronize the local population of adipocytes. For example, adipocytes exposed to hypoxia, a condition known to inhibit insulin signaling in adipocytes, release sEVs that cause insulin resistance in adipocytes cultured in normoxia [57, 58]. In addition, a study using rat adipocytes showed that large adipocytes release lEVs that are transferred to small adipocytes where they stimulate lipid accumulation and an increase cell size [59]. This is the result of lEV mRNA cargo that codes for proteins involved in fatty acid esterification, lipid droplet biogenesis, and adipokines [59]. Further work is needed to understand the extent and consequences of adipocyte-adipocyte EV transfer in energy homeostasis and metabolic disease.

Endothelial cells (ECs) are another AT tissue cell type reported to communicate with adipocytes via EVs, although this interaction is greatly understudied. We have previously shown that there is robust transfer of cellular components via EVs from ECs to adipocytes and vice versa in AT [18]. The EC-to-adipocyte transfer of EVs is enhanced in the fasted state due to glucagon-simulation of EC sEV production and attenuated in obese tissue [18]. This suggests these sEV transfer events are regulated by the systemic metabolic state. Although the signaling consequences of this EV-mediated communication is still under investigation, Wadey et al. have demonstrated that 3 T3-L1 adipocytes treated with TNFα secrete EVs that upregulate adhesion proteins in human umbilical vein ECs (HUVECs; [29]). This promotes leukocyte attachment to the ECs, a prerequisite for atherosclerotic plaque formation, which is common in obesity. Similarly, sEVs from visceral AT have been shown to exacerbate atherosclerosis in apolipoprotein E-deficient mice through activation of M1 macrophages [37], but likely also by promoting leukocyte adhesion to the vascular wall. Furthermore, sEVs isolated from insulin resistant 3 T3-L1 adipocytes enhanced vasa vasorum angiogenesis in HUVECs, a form of angiogenesis that occurs in type 2 diabetes and is associated with atherosclerotic plaque rupture [60]. Exogenous administration of sEVs from insulin resistant adipocytes reduce plaque stability in mice through Sonic hedgehog signaling [60]. These studies suggest the adipocyte-EC EV communication axis is important in the development of comorbid conditions in obesity warranting further inquiry.

6 Distal functions of adipocyte-derived EVs

Ad-EVs can signal in an endocrine manner by entering circulation. Although little is understood about Ad-EV cell targeting mechanisms, we have some evidence that Ad-EVs can influence the function of the liver, skeletal muscle, hypothalamus, heart and lungs (Fig. 3).

Liver The liver seems to be a robust signaling target of Ad-EV-associated miRNAs [4]. In the context of metabolic disease, sEVs isolated from human obese visceral AT dysregulate the TGFβ pathway in hepatocyte cell lines, a characteristic feature of non-alcoholic fatty liver disease (NAFLD; [61]). Furthermore, EVs from obese human subcutaneous or omental AT induce insulin resistance in the HepG2 hepatocyte cell line [21]. Hepatocytes also signal reciprocally to adipocytes. sEVs isolated from primary hepatocytes from mice on a high

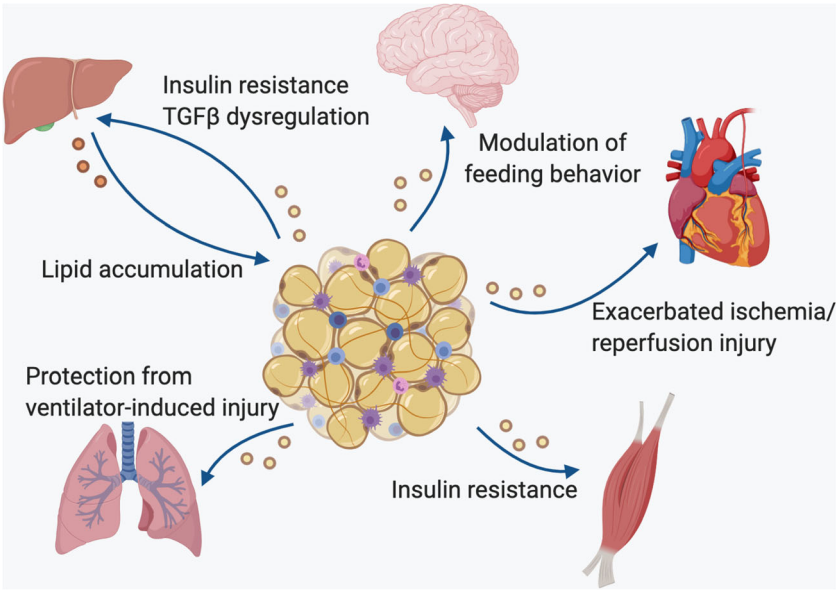

Fig. 3 AdEVs or AT EVs enter circulation and carry out signaling in distal organs. AdEV or AT EVs protect the lungs from ventilator-induced injury and induce insulin resistance in skeletal muscle and the liver. The liver releases sEVs that promote lipid accumulation in AT. AdEVs also signals to the hypothalamus to adjust food-intake and imposes pathological stress on cardiac tissue

fat diet enhance lipogenesis and lipid storage of 3 T3-L1 adipocytes [62]. Suppression of liver sEV production *in vivo* using a liver specific geranylgeranyl diphosphate synthase (Ggpps) knockout mouse resulted in reduced adipose lipid accumulation during high fat feeding [62]. These studies suggest a pathological cycle is established in obesity where EVs from the lipid-stressed liver stimulate adipose lipid storage. The hypertrophied adipocytes respond by releasing EVs that promote fibrogenic signaling and insulin resistance in the liver, which further exacerbates the liver lipid load.

Lungs sEVs isolated from human primary adipocytes are also taken up by a lung epithelial cell line A549, suggesting a role of AdsEVs in lung physiology [16]. AdsEVs from obese humans transferred miRNAs to A549 cells that regulate the TGFβ and Wnt/βcatenin pathways, both involved in inflammatory and fibrogenic signaling [16]. Based on this finding, it is surprising that visceral AT-EVs protect high-fat fed mice from ventilator-induced lung injury [63]. However, this was not likely the result of Ad-EVs, but rather adipose tissue stem cell (ADSC)-derived sEVs seemed to mediate this effect [63].

Hypothalamus To date only two studies have demonstrated EV-mediated communication between the AT and the hypothalamus. The arcuate nucleus (ARC) is a region with a leaky blood-brain barrier, so it is accessible to sEV-mediated signals [64]. Goa et al. reported that sEVs secreted by isolated visceral adipocytes from obese mice enhanced food intake and increased body weight gain in chow fed mice [64]. They found this phenotype was the result of miRNA and IncRNA transferred to POMC neurons via sEVs, where they stimulate the mTORC1 signaling pathway [64]. The opposite was also true, sEVs from adipocytes of lean mice reduce appetite and attenuated weight gain in high fat-fed mice by reducing mTORC1 signaling [64]. The second study demonstrated that circulating extracellular nicotinamide phosphoribosyltransferase (eNAMPT) was exclusively found in EVs, the presence of which is significantly reduced during aging [65]. As NAMPT is the rate-limiting step in NAD^+ synthesis, the total levels of NAD^+ decline in all tissues with age. Yoshida et al. reported that EV-associated eNAMPT could be restored in circulation by overexpression of eNAMPT in adipocytes. This resulted in improved NAD^+ biosynthesis in the hypothalamus and was associated with an increase in the lifespan of female mice [65].

Heart and skeletal muscle Ad-sEVs also induce pathological signaling in cardiac and skeletal muscle. Palmitate-treated 3 T3-L1 adipocyte sEVs induce insulin resistance in C2C12 cells via miR-27a-mediated repression of PPARγ [66]. In the case of myocardial signaling, primary adipocytes isolated from the epididymal AT and treated with high glucose and palmitate media released sEVs that exacerbated myocardial ischemia/reperfusion injury in diabetic mice [67]. This was found to be the result of miR-130b-3p-mediated suppression of anti-apoptotic and cardioprotective pathways, such as AMPK [67]. Although not discussed in this study, it would be interesting to determine the role of epicardial adipose tissue-derived EVs in this pathological mechanism. There is growing interest in the theory that dysfunctional epicardial adipose may actively contribute to the development of cardiovascular disease in obese individuals, although no EV-based mechanism has been proposed [68].

7 Future considerations

This survey of the current literature provides a compelling case that Ad-EVs actively participate in obesity-associated

pathogenic signaling within adipose tissue itself and system-wide in distal tissues. This opens an exciting avenue for therapeutic intervention in the progression of obesity and type 2 diabetes. However, our understanding of adipocyte-mediated EV signaling is still too limited to develop effective pharmacological strategies. Critical steps towards a better understanding will need to be taken to understand the fundamentals of Ad-EV signaling: *1)* future studies should focus on primary cell culture models or *in vivo* studies, as most of the data we have now is derived from immortalized cell lines; *2)* there is a need to evaluate AT depot-specific EV signaling. Many studies discussed here have focused on EVs from epididymal adipocytes. As epididymal/visceral AT is inherently more inflammatory under obese conditions, EVs from subcutaneous depots may yield different physiological outcomes; *3)* new genetic mouse models need to be developed to quantify the contribution of adipocyte EVs to the total circulating population, trace cell-specific EVs to discover target tissues, and manipulate EV production in a cell-specific manner; *4)* cell targeting mechanisms (i.e. adhesion molecules or surface receptors) need to be identified to enable targeted delivery of content to critical tissues and cell types.

The field of EV-mediated signaling in metabolic regulation is still in its infancy. We do appreciate however that this represents an entirely new chapter in endocrinology that will also have a potent paracrine component to it. With continued collaborative efforts, we can move the field towards a translatable understanding of EV biology in homeostatic and pathologic settings.

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Conflict of interest The authors have no conflicts of interest.

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