Interorgan communication by exosomes, adipose tissue, and adiponectin in metabolic syndrome

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Adipose tissue plays important roles in regulating whole-body energy metabolism through its storage function in white adipocytes and its dissipating function in brown and beige adipocytes. Adipose tissue also produces a variety of secreted factors called adipocytokines, including leptin and adiponectin. Furthermore, recent studies have suggested the important roles of extracellular vesicles of endosomal origin termed exosomes, which are secreted from adipocytes and other cells in adipose tissue and influence whole-body glucose and lipid metabolism. Adiponectin is known to be a pleiotropic organ-protective protein that is exclusively produced by adipocytes and decreased in obesity. Adiponectin accumulates in tissues such as heart, muscle, and vascular endothelium through binding with T-cadherin, a glycosylphosphatidylinositol-anchored (GPI-anchored) cadherin. Recently, adiponectin was found to enhance exosome biogenesis and secretion, leading to a decrease in cellular ceramides, excess of which is known to cause insulin resistance and cardiovascular disease phenotypes. These findings support the hypothesis that adipose tissue metabolism systemically regulates exosome production and whole-body metabolism through exosomes. This review focuses on intra-adipose and interorgan communication by exosomes, adiponectin-stimulated exosome production, and their dysregulation in metabolic diseases.

Introduction

Obesity is associated with an array of complications, including cardiovascular diseases, type 2 diabetes (T2D), fatty liver disease, osteoporosis, airway disease, degenerative disorders including dementia, and certain cancers. In fact, obesity accompanied 4.72 million deaths and 148 million disability-adjusted life-years globally in 2017. It was the fourth leading risk factor for mortality (1, 2). These complications are considered to occur in large part as a result of insulin resistance induced by obesity. Important to note is the notion that adipose tissue is not only an energy storage organ but also an endocrine organ, secreting factors that affect the function of cells and tissues/organs throughout the body. Overproduction and/or imbalance of adipose secretome, i.e., overproduction of proinflammatory adipocytokines and diminished expression of antiinflammatory adipocytokines as occurs in obesity are considered to account for systemic metabolic dysfunction and cardiovascular disease (3). In addition to such dysregulation of adipocytokines, exosomes secreted from adipocytes and other cells in adipose tissue have been revealed to be altered in obesity (4–7). Exosomes are vesicles that envelop intracellular molecules including microRNAs, bioactive lipids, and proteins (8, 9). Their potential roles in cell-to-cell communication position them as previously unrecognized and important endocrine machinery.

Among the various adipocytokines, adiponectin exists in plasma at the highest circulating concentration (10–13). It circulates at between 3 and 30 μg/mL in plasma, corresponding to molecular quantities that are 10^3- to 10^6-fold higher than ordinary hormones and cytokines. Since its discovery as the most abundant adipose secretory protein around 20 years ago (10–13), adiponectin has appeared in the title of over 7,000 reports. However, two very important questions remained unanswered until now: (i) Why is such an abundant concentration of adiponectin in plasma required? (ii) Why are multimeric conformations required for adiponectin to exert its functions?

Several recent studies have reported that T-cadherin mediates cardiovascular protection and skeletal muscle regeneration via the clinically important high molecular weight (HMW) multimeric adiponectin (14–17). T-cadherin facilitates adiponectin accumulation in multivesicular bodies, a subtype of endosome in which exosomes are generated, wherein adiponectin stimulates exosome biogenesis and secretion (17–19).

In this Review, we will discuss adipose tissue exosomes, their roles as signaling packages and waste-disposing vesicles, and further, their roles in glucose and lipid metabolism and adiponectin functions in exosomes. We also discuss the current technological limitations of studying exosome and future perspectives.

Adipose tissues secrete adipocytokines and exosomes

Adipose tissues secrete exosomes, influencing whole-body metabolism. It is recognized that adipose tissue is the largest endocrine organ, secreting over 600 proteins (20–22), including the first-discovered leptin (23) and the subsequently described adiponectin (10–13). To date, numerous adipocyte-derived secretory factors have been identified that play a role in the maintenance of glucose, lipid, and energy homeostasis, each of them contributing to communication

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Table 1. Evolutionary view of adipose tissue–, exosome-, and adiponectin-related proteins

	Vertebrates							
	Human	Mice	Birds	Frogs	Fish	Drosophila	Nematode	Yeast
Fat storage site	WAT	WAT	WAT	WAT	WAT	Fat body	Intestinal cells	Droplets
Leptin	Yes	Yes	Yes	Yes	Yes	No	No	No
Adiponectin	Yes	Yes	Yes	Yes	Yes	No	No	No
T-cadherin	Yes	Yes	Yes	Yes	Yes	No	No	No
CALR	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
AdipoR1/R2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Exosomes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Energy storage in the form of triglycerides in droplets is an evolutionarily conserved way to keep energy in the form of biologically inactive material in a separate repository for future needs (26). For example, lipid droplets are conserved from yeast to mammals (136), and the droplet-stabilizing perilipin family (PLIN family) protein also exists in yeast (137) and *Caenorhabditis elegans* (138). Fat is stored in the intestine in *C. elegans*, a defined fat body in Drosophila. Sharks lack adipose tissue and use their liver to store fat, although their genomes have homologs of leptin and adiponectin. Exosomes, too, are conserved among eukaryotes and produced in essentially all cells throughout the body (27). WAT becomes apparent in vertebrate species, coinciding with the appearance of leptin, adiponectin, and T-cadherin (26, 139). APN, adiponectin; T-cad, T-cadherin; calr, calreticulin. The table is adapted in large part from a previous publication (26), combined with knowledge based on genetic information from the NCBI Gene database.

within adipose tissues and/or interorgan communication between adipose tissue and other tissues involved in the maintenance of metabolic homeostasis, such as muscle, heart, liver, islets, kidney, and even brain. Moreover, recent understanding of the secretory role of adipose tissues went beyond such secreted proteins. Thomou et al. reported that the majority of circulating microRNAs included in exosomes are derived from adipocytes and travel to the liver, where they improve glucose tolerance and reduce hepatic *FGF21* expression (7). Crewe et al. demonstrated exosomal communication between endothelial cells and adipocytes in adipose tissues (24). They elaborately isolated adipose-tissue extracellular vesicles and showed that proteins and lipids capable of modulating cellular signaling pathways were involved in such small vesicles (24). Flaherty et al. reported that adipocytes can release neutral lipids directly via exosomes in addition to the lipolysis reaction, which releases fatty acids and glycerol through the action of neutral lipases (4). Ying et al. demonstrated that exosomal microRNAs derived from adipose tissue macrophages regulate whole-body glucose metabolism by modulating adipocyte functions (6). Pan et al. reported that adipocyte-secreted exosomal microRNA-34a inhibits M2 macrophage polarization to promote obesity-induced adipose inflammation (5). Together, these studies support the broad influence of adipose tissue–derived exosomes in whole-body metabolism.

Evolutionary view of adipose tissue, exosomes, and adipocytokines. Energy storage in the form of triglycerides is a conserved way to store energy for future needs. Although sharks store fat primarily in the liver (25), a tissue of endodermal origin, most vertebrate species store fat primarily in white adipose tissue (WAT), a tissue of mesodermal origin (26) (Table 1). From an evolutionary viewpoint, WAT's appearance in vertebrates accompanied a dramatic change in organ fat storage, from simple fat storage to an organ that secretes adipocytokines like leptin and adiponectin (Table 1). However, exosomes, defined as secreted vesicles generated in late-endosomal compartments, are conserved among eukaryotes (27).

A striking difference between mammals and other vertebrate animals is their diverse distribution of WAT in many areas in the body, including intra-abdominal depots around the visceral organs, intestines, and perirenal areas, as well as in subcutaneous depots in the buttocks, thighs, and abdomen (26). In mammals, the adipose organ is composed not only of white adipocytes, which are the primary site of energy storage, but also brown adipocytes, which are important in energy expenditure in the form of thermogenesis. Adiponectin is expressed, in this sense, throughout the body, irrespective of adipose tissue color, i.e., in every adipocyte in all areas, including in beige/brown adipocytes, in ectopic adipocytes that are occasionally observed in degenerating tissues, and in bone marrow adipocytes (28, 29). Such ubiquitous expression of adiponectin in all adipocytes lets the adiponectin promoter be exploited to target adipocyte-specific expression of genes of interest, using Cre recombinase or other methods, to produce adipocyte-specific transgenic mice.

Three types of adiponectin binding partners have been suggested: adiponectin receptors (adipoRs), calreticulin, and T-cadherin. From an evolutionary viewpoint, WAT's appearance in vertebrates accompanied the emergence of both adiponectin and T-cadherin (Table 1).

Exosomes are multifunctional in glucose and lipid metabolism

Exosome biogenesis and secretion. Exosomes initially form as intraluminal vesicles (ILVs), which are generated by inward budding of the limiting membrane during endosome maturation into multivesicular bodies (MVBs) in the endocytic pathway (30). The endosomal sorting complexes required for transport (ESCRT-0, -I, -II, -III) and the associated ATPase Vps4 complex play important roles in this process. However, after depletion of key subunits of all four ESCRTs, ESCRT-independent ILV biogenesis still exists in mammalian cells. MVBs can fuse either with lysosomes for intracellular degradation or with the plasma membrane, resulting in release of ILVs into the extracellular space as exosomes (27).

Exosome biogenesis and/or release is affected by various molecules, including the ESCRT machinery components, Rab GTPases acting on vesicular traffic, membrane-spanning tetra-

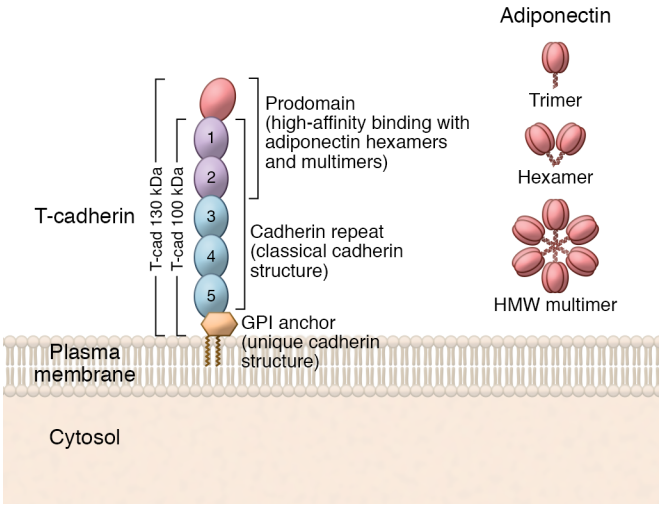

Figure 1. Structure of T-cadherin. T-cadherin is a unique GPI-anchored cadherin. It is classified as a member of the classical cadherins, but it lacks C-terminal transmembrane and cytosolic domains (106). T-cadherin exists both on the plasma membrane and in endosomes, including multivesicular bodies where exosomes are generated (18). T-cadherin was discovered as an adiponectin binding partner by evaluating cell binding to coated recombinant adiponectin produced in HEK293 mammalian cells (57). Its unique prodomain gives T-cadherin high-affinity interaction with native HMW adiponectin in serum (19).

spanins, and the intracellular adaptor syntenin (31). Features of cellular metabolic status such as ceramide metabolism, ER stress, autophagy, and intracellular calcium also affect exosome biogenesis and/or secretion. Adiponectin, a protein secreted exclusively from adipocytes, stimulates exosome biogenesis in endothelial and skeletal muscle cells expressing T-cadherin (18). Crewe et al. discovered that glucagon regulates exosome production from endothelial cells in adipose tissues (24). These findings indicate that exosome biogenesis is a metabolically regulated process in addition to being a process that impacts metabolism.

Exosomes function as signaling packages. Exosomes are small vesicles (30–100 nm) delimited by a lipid bilayer and are thought functionally to mediate cell-to-cell communication under normal and pathological conditions by transferring active proteins, mRNA, and small noncoding RNAs stably in various biofluids (8, 9). In this regard, a recent study reported that adipose-derived circulating microRNAs in exosomes regulate important metabolic gene expression in distant tissues such as liver (7). DICER cleaves double-stranded RNA (dsRNA) and pre-microRNA into short, single-stranded RNA fragments called small interfering RNA (siRNA) and microRNA, respectively. The study by Thomou et al. found that circulating microRNA content was decreased in adipocyte-specific *Dicer*-KO mice, suggesting that adipocyte-secreted exosomes occupy the majority of circulating exosomes (7). In contrast, however, using adipocyte-specific expression of the fluorescent protein tdTomato in mice, Flaherty et al. reported that adipocyte-derived exosomes represent a minority of the exosomes in the circulation under normal conditions (4). Despite these apparently conflicting findings, a number of studies (discussed below) support the importance of exosome-derived signals from adipose tissue in energy homeostasis and metabolism.

In clinical studies, it was reported that exosomal protein and microRNA profiles were altered in T2D patients (32, 33). In accordance with these human data, it was reported that adipose tissue macrophage–derived exosomes affected insulin sensitivity in mice (6). Exosomes from adipose tissue macrophages in obese mice caused glucose intolerance and insulin resistance when administered to lean mice (6). In turn, exosomes from adipose tissue macrophages in lean mice improved glucose intolerance and insulin resistance when administered to obese mice (6). The changes observed in microRNAs involved in exosomes, such as miR-155, were suggested to have important roles in regulating insulin sensitivity and glucose homeostasis (6). Moreover, an obesity-associated increase of exosomal microRNA-34a secretion from adipocytes inhibited M2 macrophage polarization and promoted obesity-induced adipose inflammation (5). The expression of miR-34a in adipose tissues also progressively increased with the development of diet-induced obesity in mice. Adipose- or adipocyte-specific miR-34a–KO mice were resistant to obesity-induced glucose intolerance, insulin resistance, and systemic inflammation, and this was accompanied by antiinflammatory M2 polarization of adipose-resident macrophages from the proinflammatory M1 phenotype (5). Taken together, these findings suggest the important roles of exosomal transfer of microRNAs for the regulation of glucose and energy metabolism (4–7).

On the other hand, a quantitative assessment of the microRNA content of extracellular vesicles using a stoichiometric approach showed that microRNA copy number per vesicle in a blood sample was less than 1, even for the most abundant microRNAs, suggesting that most individual exosomes do not carry biologically significant numbers of microRNA and are unlikely to function as vehicles for microRNA-based communication (34). Because an exosome is a package of multiple bioactive proteins, lipids, and nucleic acids including microRNAs, its function in the recipient cells might be based on the orchestration of a variety of signals.

A recent report noted increased particle numbers in fasting plasma of T2D patients versus euglycemic controls (35). Quantification of exosomes in biological fluids is still technically challenging (36, 37). Polyethylene glycol–mediated precipitation methods like ExoQuick used in this study precipitate essentially all large molecules, including lipoproteins and aggregated proteins, in addition to exosomes and microvesicles. Problematically, nanoparticle tracking analysis (NTA) cannot discriminate them; it can only evaluate the size distribution of particles in test fluids. A simple, semiquantitative method was developed to measure exosomes in blood based on the combination of polyethylene glycol–based purification, standard differential ultracentrifugation techniques, and Western blotting (18, 38). Although it is semiquantitative, this method can measure a number of exosome components simultaneously and may give information on changes in some important exosome cargos induced by metabolic challenges.

Recently, adipose tissue endothelial cells were reported to produce high amounts of exosomes in response to glucagon (24). This study established the importance of exosome-mediated intra-adipose and interorgan communications in energy metabolism. Exosomes secreted from adipose endothelial cells travel to neighboring adipocytes, and reciprocally, adipocyte-secreted exosomes travel to endothelial cells. These exosomes can be taken up by neighboring macrophages in adipose tissue, and are

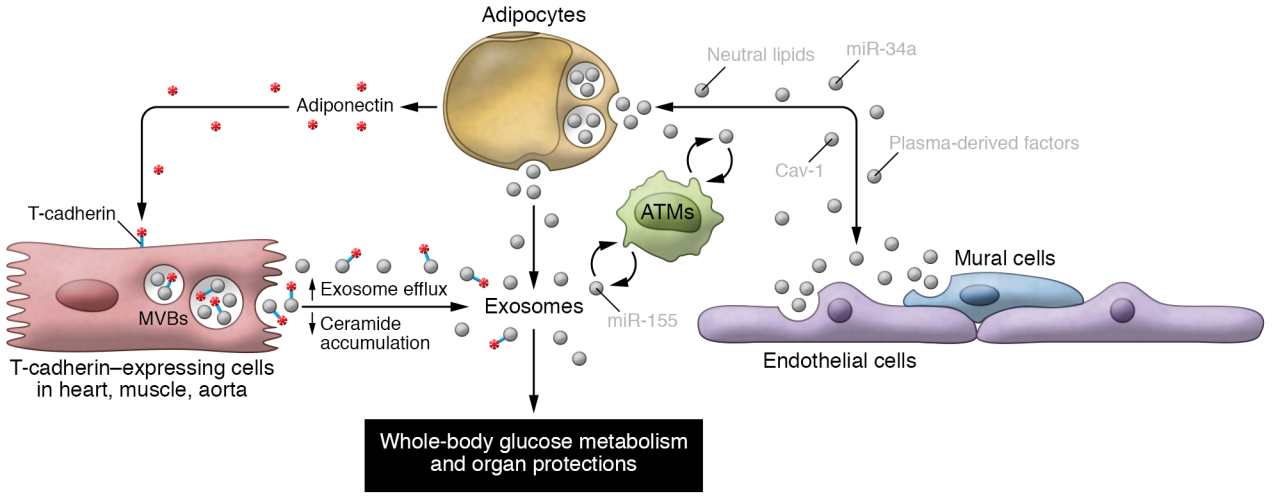

Figure 2. Exosome-mediated transfer of signals and substances controls whole-body glucose metabolism. Various cell types communicate with each other by transferring signals, such as microRNAs, and substances, such as neutral lipids in adipose tissue (4–6, 24). Macrophages phagocytose and secrete exosomes. Adipose tissue–derived exosomes affect whole-body glucose metabolism (6, 7). Adiponectin secreted from adipocytes stimulates exosome production from T-cadherin–expressing cells in aorta, heart, and muscle and affects systemic exosome levels (17–19). The adiponectin-mediated production of exosomes also may also affect whole-body glucose metabolism.

rich in proteins and lipids that are capable of modulating cellular signaling pathways (24). In agreement with this notion, adipocyte-derived exosomes containing large amounts of lipids induced an adipose tissue macrophage–like phenotype in bone marrow cells in cell culture (4). It is noteworthy that these two studies elaborately developed new methods to isolate adipose tissue extracellular vesicles (24) and adipocyte-derived exosomes (4), respectively. Moreover, these novel and fundamental notions might challenge the long-believed "adipose chronic inflammation theory" that is based on observations that increased lipolysis activates macrophages. Therefore, it may be reasonable to speculate that metabolically stressed adipocytes in obesity may secrete exosomes bearing information that triggers low-grade inflammation, thus causing adipose inflammation and insulin resistance.

Exosomes function as waste disposal bags. In addition to the above-mentioned signaling role of exosomes, exosomal release serves as an alternate disposal pathway to lysosomes (39). Misfolded proteins or several aggregated toxic proteins, such as amyloid β (40), tau (41), and prion protein (42), were shown to be released in exosomes. A more recent study revealed that a cell's exosomal release suppresses its own cellular senescence by excreting accumulated harmful cytoplasmic damaged DNA (43). These observations suggest that a cell's exosomal release can attenuate its own cellular stress and maintain its homeostasis by exporting various unnecessary or harmful materials.

Although the fate of secreted exosomes and their contents is important, the molecular basis for their active targeting to acceptor cells is not well understood. For example, it is reported that integrins on exosomes can interact with adhesion molecules and matrix proteins on acceptor cells, thus enabling active targeting (44). How bound exosomes release their content, i.e., microRNAs and bioactive proteins, into the cytosol of the recipient cells for signaling is poorly understood. Recent studies suggested the possible existence of a mechanism by which RNA and other exosome cargos can escape the intrinsically degradative endosomal pathway (45–47). On the other hand, the majority of exosomes can be phagocytosed by macrophages (48–51). Exosomes have no intrinsic ATP production and expose phosphatidylserine on their surface, similarly to apoptotic cells, thus enabling their recognition by phagocytes like macrophages (49, 51–53). Phagocytosed exosomes travel into late endosomes, including MVBs, and large parts can be degraded by lysosomes in macrophages, which have more degradative activity than other cells. Interestingly, adipocyte-derived exosomes were found to contain large amounts of neutral lipids and induce lipid-laden macrophages in adipose tissue (4). Such lipid droplets in macrophages are catabolized, not by an autophagy-related pathway but by an endocytosis-related pathway, suggesting that the droplets are not generated within macrophages but rather are endocytosed into macrophages (4, 54). Thus, the tissue-specific phenotype and function of macrophages in adipose tissue may be induced by fatty acids generated in lysosomes from such neutral lipids transferred from adipocytes via these atypical exosomes.

Adiponectin regulates systemic exosome level through T-cadherin

There have been three classes of adiponectin receptor reported: adipoRs (55), calreticulin (56), and T-cadherin (57). Although adiponectin was reported to exert metabolically important roles through adipoRs (58–60) and immunologically important roles through calreticulin (57), our review will primarily focus on T-cadherin because of the large body of evidence supporting its modulation of adipose exosomal communication.

Adiponectin circulates in plasma in three major forms: trimer, hexamer, and HMW multimer (61–63). The HMW multimeric adiponectin has been shown to be the more active form of adiponectin in improving insulin sensitivity and protecting against glucose intolerance (64–67). Impaired multimerization or selective reduction of HMW multimeric adiponectin concentrations in plasma was also associated with various metabolic diseases such as obesity, insulin resistance, T2D, and arteriosclerosis (67–69). Increase

in the ratio of HMW multimeric adiponectin to total adiponectin was more closely associated with improved insulin sensitivity in diabetic mice and PPARγ agonist–treated diabetic patients than total adiponectin (69), further supporting the HMW multimer's physiological relevance.

Obesity, aging, and heart failure regulate plasma adiponectin. HMW octadecameric adiponectin exceeds a molecular size of 450 kDa (70). After secretion from adipocytes into the adipose interstitial fluid, this large multimeric adiponectin presumably cannot pass through the capillary wall into circulation; rather, it is likely to enter the lymph from interstitial fluid (71). Because lymph transport takes longer and variable time to enter into circulation relative to capillary transcytosis, this notion is compatible with the observation of relatively little diurnal change in plasma adiponectin concentration in human subjects (72), despite the profound change in expression and secretion seen in cultured adipocytes following metabolic challenges (10, 73).

Once secreted from adipocytes, there seems to be no active interconversion between molecular species of adiponectin in plasma (61). The most striking feature of this beneficial adipocytokine is its decrease in obesity in human subjects. Adiponectin circulates at the highest plasma concentration known among adipocytokines (32, 74–76). In humans, plasma adiponectin concentration was inversely correlated with body weight and BMI in cross-sectional research (76, 77). Regulation of adiponectin's circulating level has not been fully elucidated, although negative-regulation mechanisms have been demonstrated, such as those involving TNF-α (78), IL-6 (79), ROS (80, 81), androgen (82), and aldosterone (83, 84), all of which were shown to associate with obesity.

Plasma adiponectin level is inversely associated with BMI; on the other hand, it is positively associated with age, even after adjustment for visceral adiposity (85, 86). The decrease in its clearance rate in the kidney may be the cause of high levels of adiponectin in the elderly (87, 88). In extreme leanness, increased production from increased bone marrow adipose tissue combined with increased production from WAT contributes to hyperadiponectinemia (28). Bone marrow adipose tissue also increases with aging and may account for a higher level of adiponectin in elderly people. On the other hand, C57BL/6J mice, the strain usually used for metabolic experiments, have very few bone marrow adipocytes, especially at young ages (29); thus, they may not be able to represent the changes of adiponectin in plasma associated with aging and obesity. In agreement with this, the well-known inverse association of weight and plasma adiponectin levels in human subjects was not observed consistently in rodent models (89, 90).

Several clinical studies have suggested that plasma adiponectin level is an independent prognostic predictor in patients with chronic heart failure (CHF), naming this the "adiponectin paradox" (48, 91–93). In a prospective study, increased plasma adiponectin predicted poor outcome in future cardiovascular events in patients presenting with stable angina and preserved systolic function (94). Although it is possible that elevated natriuretic peptides in CHF patients may increase adiponectin levels (95), beta blocker use may also play a role in regulating adiponectin (96, 97). Interestingly, a decrease in plasma adiponectin concentration was significantly correlated with improvement of cardiac function by beta blocker treatment (96). As we discuss below, organ protection by adiponectin may require T-cadherin and consumption of adiponectin through T-cadherin binding (14–16, 98). Low tissue T-cadherin and/or low T-cadherin–mediated consumption of adiponectin may result in poor CHF prognosis.

Physiological roles of adiponectin, genetic studies in mice and human subjects. A comprehensive multiethnic analysis of the genetic influences on adiponectin levels was performed and yielded 12 loci, including *ADIPOQ* (encoding adiponectin) and *CDH13* (encoding T-cadherin) (99). A Mendelian randomization approach also tested the hypothesis that adiponectin causally influences insulin resistance and T2D and found no evidence associating adiponectin-lowering alleles with insulin sensitivity and T2D (100), or with coronary heart disease (101). Mendelian randomization has been increasingly used in clinical research and is a powerful tool to help understand mechanisms of disease, especially given the complex metabolic phenomena that commonly occur in human diseases. These studies indicate that SNPs nearby or in the *ADIPOQ* locus that affect plasma adiponectin level have little impact on insulin sensitivity, diabetes, and cardiovascular disease risk. On the other hand, a multi-loci set of SNPs (liberal approach; gathering all SNPs affecting plasma adiponectin level together) revealed a significant association with cardiovascular disease risks, suggesting that SNPs outside of the *ADIPOQ* locus that affect plasma adiponectin levels may be important for cardiovascular disease risk (101). Because SNP-associated variation in plasma adiponectin levels in human subjects must be smaller than those induced by a genetic deficiency of adiponectin in mice, animal studies may reveal larger consequences of adiponectin loss. Furthermore, SNPs' small effects on plasma adiponectin levels might be compensated for during life-long periods in human subjects. Indeed, it was reported that the acute depletion of adiponectin resulted in more severe systemic insulin resistance and hyperlipidemia than in mice with congenital loss of adiponectin (102).

Outside of the *ADIPOQ* locus, the gene locus that most strongly affects plasma adiponectin levels in GWAS is *CDH13* (103). Several SNPs in the *CDH13* locus are associated with both cardiovascular disease risks and glucose homeostasis (103, 104). Importantly, SNPs in the *CDH13* locus are also reported to associate with higher plasma adiponectin levels and higher odds ratio for cardiovascular disease risks simultaneously in human subjects (101, 105). Loss of T-cadherin in mice significantly increased plasma adiponectin level, and in turn, adiponectin increased T-cadherin protein expression in aorta, heart, and muscle in mice (14, 98). Thus, both genetically modified mice and human genetic analyses suggest that adiponectin's actions are mediated through T-cadherin.

T-cadherin. T-cadherin is a unique member of the cadherin family of cell adhesion molecules, possessing a GPI anchor instead of a transmembrane and intracellular domain (106) (Figure 1). It is classified as a member of classical cadherins like E-cadherin and N-cadherin by its high homology of 5 extracellular cadherin repeats. However, the GPI anchor on T-cadherin's C-terminus differentiates it from other cadherins (Figure 1).

T-cadherin was cloned by expression cloning from a C2C12 myotube cDNA library by evaluating cell binding to recombinant adiponectin produced in HEK293 mammalian cells (57). T-cadherin binds multimeric adiponectin, including the hexameric form and larger multimers (19, 57).

Clinical analyses have demonstrated that HMW multimeric adiponectin is the active form, and it exerts various pleiotropic effects (64–67). Recent studies have shown that the active HMW multimers and hexamers of adiponectin accumulate in tissues such as heart, vascular endothelium, and skeletal muscles through interaction with T-cadherin (14–17, 98). Plasma adiponectin increased more than 3-fold in T-cadherin–KO mice compared with WT mice, with a dramatic increase of HMW multimeric adiponectin (98). Importantly, several reports have shown the importance of adiponectin association with T-cadherin in organ protection in models of cardiac hypertrophy (14), hind limb ischemia (15), atherosclerosis (16), and skeletal muscle regeneration (17). Furthermore, as discussed above, SNPs near the T-cadherin gene correlate strongly with plasma adiponectin level and cardiovascular disease risk in human subjects (101, 103, 105, 107–112).

T-cadherin is an atypical cadherin with a GPI anchor, and without the transmembrane and intracellular domains required for typical signaling, which has long hindered reaching the conclusion that T-cadherin is the receptor for adiponectin. However, a recent study established a high-affinity interaction between T-cadherin and native HMW multimeric adiponectin purified from mouse serum (19). T-cadherin fused with human IgG Fc as a fusion tag replacing its GPI anchor specifically bound both hexameric and HMW multimeric adiponectin in serum, with a dissociation constant of approximately 1.0 nM and without contribution from other cellular or serum factors (19). The extracellular T-cadherin repeats 1 and 2, which overlap with the regions required for classical cadherin-mediated cell-to-cell adhesion, were critical for the observed adiponectin binding (19) (Figure 1). Moreover, the 130-kDa prodomain-bearing T-cadherin, uniquely expressed on the cell surface among members of the cadherin family, was predominantly increased by adiponectin and significantly contributed to adiponectin binding (19) (Figure 1).

Adiponectin regulates exosome biogenesis and systemic exosome levels. Exosome biogenesis stimulation by adiponectin was the first demonstration that this secreted factor can modulate exosome biogenesis and secretion (18) (Figure 2). Adiponectin was detected in intracellular compartments in endothelial cells of WT mouse aorta by ultrastructural immune-electron microscopy analysis (113). Adiponectin together with T-cadherin accumulated inside multivesicular bodies, the site of exosome generation, both in cultured endothelial cells and in vivo in WT mouse aorta (18). Adiponectin also accumulated in exosomes in the conditioned media of endothelial cells that were cultured with native adiponectin from mouse serum and even in exosomes in the blood of WT mice, but not in T-cadherin–deficient mice (18). The systemic level of exosomes in blood was decreased almost half by genetic loss of adiponectin or of T-cadherin and increased by overexpression of adiponectin in mice (18). Adiponectin existing in serum or purified from serum rich in HMW multimeric adiponectin stimulated exosome production from cultured endothelial cells (18). The exosomal effect of HMW multimeric adiponectin was dose dependent in at least the physiological concentration range (18). Adiponectin-induced increases in exosome biogenesis in cultured cells were dependent on T-cadherin (18), and were not restricted to cultured endothelial cells but also observed in C2C12 differentiated myotubes (17) (Figure 2). Because T-cadherin is expressed broadly in muscle, heart, and vessels, and adiponectin affects systemic exosome levels, the decreased microRNAs in serum exosomes in *Dicer*-KO mice may be the result of extremely reduced adiponectin and adiponectin-mediated exosome biogenesis in these lipodystrophic mice (7, 18).

It has been shown that higher-order oligomerization of protein with membrane anchoring is sufficient for protein sorting into the exosome (114). Furthermore, clustering of an extremely abundant membrane protein, syndecan, via association with the intracellular adaptor syntenin was reported to stimulate exosome biogenesis in a cell culture model (115). Adiponectin, with its multimeric structure, may cause higher-order clustering of T-cadherin, a membrane-anchored protein.

In accordance with the above-mentioned signaling and cellular maintenance roles of exosomes, T-cadherin can transmit adiponectin signals both into T-cadherin–expressing cells, such as endothelial, muscle, and cardiac cells and to neighboring or distant cells by enhancing secretion of exosomes into extracellular fluids and circulation (Figure 2).

Relating to the cellular maintenance role of exosomes on exosome-producing cells, adiponectin stimulated ceramide efflux to exosomes and decreased cellular ceramide levels (18). Ceramide accumulation in cells is known to induce apoptotic pathways (116, 117) and attenuate insulin sensitivity (118, 119). Disposal of ceramides to macrophages via exosomes may constitute an intercellular and/or interorgan metabolic system of transferring ceramides to macrophages, whose lysosomal functions are much more extensive than other cell types. Overaccumulation of ceramides specifically in macrophages in Farber disease patients who lack lysosomal ceramidase may also indicate the physiological importance of this exosomal ceramide transport to, and degradation in, macrophages (120).

Regarding adiponectin, we return to two important questions mentioned in the Introduction that remain unanswered: (i) Why are such abundant concentrations of adiponectin in plasma required? (ii) Why are large multimeric conformations of adiponectin required for exerting its functions? We suggest that the exosome-stimulating function of adiponectin may partly answer these questions.

Future perspectives

Technical advances required for studying exosomes' functional role. Currently, technical advances in studies on extracellular vesicle are still lacking, specifically approaches for isolation and discrimination of types of vesicles, i.e., exosomal vesicles of endosomal origin, microvesicles, or ectosomal vesicles of plasma membrane origin, and others such as apoptotic bodies and artifactual particles released from cells broken during experimental processing (121). Lack of sophisticated technology to create loss-of-exosome animals, even restricted to organs or specific types of cells in living animals, limits studies of exosome-mediated processes. For example, inhibition of neutral sphingomyelinase 2 (nSMase2) by the small compound GW4869 is commonly used to block exosome biogenesis. However, nSMase2 is involved in cellular growth, stress response, and inflammation signaling, and its deficiency causes numerous abnormalities in mice (122). Loss of a specific microRNA will affect its producing cells as well as

the recipient cells of the exosomal transfer. Measurement of the amounts of exosome in biological fluids is still challenging; light-scattering technologies cannot even discriminate extracellular vesicles from protein aggregates (121), and quantification of total protein or lipid is overestimated as a result of contaminations (121). Technical advances in purification and quantification of exosomes in biological fluids, and in strategies to block exosome production or uptake by recipient cells in animals, will give clearer understanding of the pathophysiological roles of exosomes in metabolic syndrome.

Possible metabolic roles of adiponectin-mediated exosome production. Recent evidence indicates that adiponectin stimulates exosome biogenesis by being endocytosed into endosomes through T-cadherin binding (18). Over 20 years of adiponectin studies suggest that this adipocytokine positively affects insulin sensitivity in addition to providing cardiovascular protection (123–125). Importantly, overexpression of adiponectin in WT mice usually produces stronger effects than adiponectin deficiency. For example, muscle regeneration was improved by adenoviral overexpression of adiponectin, whereas the only tendency toward muscle attenuation was observed in adiponectin-KO mice (17). One important approach may be the application of such adiponectin functions to the development of future medications.

Depending on their source, exosomes are known to influence insulin sensitivity (5, 6, 24, 126) and cardiovascular functions (127–130). How adiponectin, especially HMW adiponectin, improves insulin sensitivity and cardiac functions through exosomes, and how this pathway can be stimulated for future therapeutics are yet to be investigated. Little has been studied regarding the role of adiponectin mediated through T-cadherin in glucose metabolism and insulin sensitivity in mice. In fact, insulin sensitivity was only evaluated under the chow-diet condition in T-cadherin–KO mice (131). Macrophages are well known to influence inflammation and insulin sensitivity (132–135) and these cells phagocytose the majority of exosomes (49, 51–53). Thus, pharmacological strategies to improve the function of the adiponectin/T-cadherin/exosome/macrophage axis may facilitate the reduction of metabolic inflammation and improvement of insulin sensitivity.

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