Exosomes as new players in metabolic organ cross-talk

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ABSTRACT

Blood glucose homeostasis requires a constant communication between insulin-secreting and insulin-sensitive cells. A wide variety of circulating factors, including hormones, cytokines and chemokines work together to orchestrate the systemic response of metabolic organs to changes in the nutritional state. Failure in the coordination between these organs can lead to a rise in blood glucose levels and to the appearance of metabolic disorders such as diabetes mellitus. Exosomes are small extracellular vesicles that are produced via the endosomal pathway and are released from the cells upon fusion of multivesicular bodies with the plasma membrane. There is emerging evidence indicating that these extracellular vesicles play a central role in cell-to-cell communication. The interest in exosomes exploded when they were found to transport bioactive proteins, mRNAs and miRNAs that can be transferred in active form to adjacent cells or to distant organs. In this review, we will first outline the mechanisms governing the biogenesis, the cargo upload and the release of exosomes by donor cells as well as the uptake by recipient cells. We will then summarize the studies that support the novel concept that miRNAs and other exosomal cargo components are new important vehicles for metabolic organ cross-talk.

KEY WORDS: Exosomes, miRNAs, cell-to-cell communication, metabolism, diabetes

INTRODUCTION

In modern societies, the combination of sedentary lifestyles with excessive caloric intake has resulted in a dramatic rise in the incidence of chronic metabolic diseases and in the associated premature deaths. Thus, it is becoming urgent to find novel strategies to cure or prevent the development of these diseases and to stop their epidemic progression. To achieve this goal, we need to improve our basic knowledge of the biological mechanisms governing the maintenance of glucose homeostasis.

Several organs contribute to the regulation of blood glucose levels and the communication between them is vital to achieve this goal. Different circulating factors including hormones, cytokines, chemokines and growth factors are known to play an important role in inter-organ crosstalk. However, recently the existence of a new cell communication mode has emerged. This new signaling mechanism is mediated by exosomes, small extracellular vesicles that are produced via the multivesicular endosomal pathway and are released by almost all cells [1-3]. Several studies performed *in vitro*, but most recently also *in vivo*, have now demonstrated that exosomes can transfer genetic material between different organs, resulting in functional changes in the receiving cells. In this review, we will discuss this novel cell-to-cell communication mode putting a particular focus on the implication of exosomal miRNA transfer in metabolic organ cross-talk.

EXOSOMES

Exosomes were identified more than 30 years ago in reticulocytes [4] and were first thought to participate in the selective removal of unwanted cell components. However, the interest in these extracellular vesicles exploded the last decade, when they were found to transport bioactive

molecules, including proteins and nucleic acids, which can be transferred in active form to recipient cells [5, 6]. Exosomes are now seen as vehicles of a new cell-to-cell signaling pathway whose rules and limitations remain to be fully established. Also, a consensus is still lacking about the nomenclature and the most appropriate isolation method of exosomes and other types of microvesicles released by the cells. Typically, the term “exosome” is used to designate extracellular vesicles (EVs) with a diameters of 50-150nm that originate from the late endosomal pathway and are released in the extracellular space upon fusion of multivesicular bodies (MVBs) with the plasma membrane [2]. Larger EVs with a diameter of up to 1000nm are generally formed by direct budding of the plasma membrane and include a wide range of membrane-enclosed entities such as ectosomes, microparticles and microvesicles. Different methods are currently used to isolate exosomes from the culture media or from serum/plasma samples. These methods discriminate the EVs based on their size, density or by the presence of specific protein components and are therefore yielding preparations with different purities and microvesicle composition. Pro and cons for each method have been extensively reviewed elsewhere [7]. The most common strategy used to isolate exosomes remains differential centrifugation. Therefore, unless specified, in this review we will focus mainly on studies on exosomes (also called exosome-like microvesicles or small EVs) that were isolated by high speed ultra-centrifugation.

Exosome biogenesis

The biogenesis of exosomes is tightly coupled to the formation of MVBs by the endocytic pathway [1, 8] (Fig.1). In brief, internalization of extracellular ligands or cellular components are processed through the endosomal pathway for their recycling to the membrane surface or for their degradation. During the endosome maturation process, several small vesicles are formed by inward budding of the early endosomal membrane, sequestering proteins, lipids and cytosolic components

that are specifically sorted. These vesicle-containing organelles are called MVBs. The main fate of MVBs is to fuse to lysosomes for degradation, but a subset of MVBs can merge with the plasma membrane, resulting in the release of the intraluminal vesicles, including exosomes, in the extracellular space (Fig.1). The mechanisms controlling the fate of MVBs are still not well understood. Specific stimuli that promote autophagy such as starvation or the inhibition of the mTOR pathway, favor the fusion of MVB with lysosomes, resulting in a decrease in exosome release [9]. ISGylation, an ubiquitin-like modification driven by ISG15 conjugation, is also involved in this process. Indeed, ISGylation of the MVB protein TSG101 induces its aggregation, favoring its lysosomal degradation and the inhibition of exosome release [10].

Exosome secretion

The best-described mechanism for the formation of intraluminal vesicles inside MVBs and for the release of exosomes involves the Endosomal Sorting Complexes Required for Transport (ESCRT) proteins. Thirty different ESCRT proteins assemble in four complexes which bind to the outer membrane of endosomes during the formation of the MVBs [1]. Their involvement in exosome formation was first suggested by proteomic studies where two ESCRT proteins, namely Alix and TSG101, were detected in exosomes of different cell types [11]. Reduction of Alix expression in tumor cells or depletion of TSG101 in HeLa or dendritic cells were found to impair exosome release [12, 13]. In other cell types, ESCRT-independent mechanisms were also observed. Inhibition of the enzyme neutral sphingomyelinase in oligodendroglial or in tumor cell lines reduced exosome secretion [5, 14], whereas in human melanoma cells, exosome release was linked to the tetraspanin protein CD-63 [15]. The fibroblasts from patients affected by the Charcot-Marie-Tooth disease have been shown to express a mutant form of the membrane protein SIMPLE and to secrete less exosomes containing CD-63 and ALIX [16]. Other regulatory molecules have also

been reported to be involved in the release of exosomes, including the Rab GTPases Rab27a and Rab27b [17] and the tumor suppressor protein p53 [18]. Thus, at present it is still unclear if a common regulatory network controls the release of exosomes in all cell types or if specific sets of molecules regulate exocytosis of different subset of exosomes depending on their formation and/or composition.

Exosome composition and miRNA cargo

Numerous studies have analyzed the protein, lipid and nucleic acid content of exosomes released by a variety of cells. The plethora of information obtained led to the creation of databases that provide catalogues of the EV content in different cell types: EVpedia [19], Vesiclepedia [20] or Exocarta [21]. The last version of Vesiclepedia (September 2015) includes data from 538 studies covering 33 species.

The mechanisms responsible for the upload of the exosomal cargo remain largely unknown. Part of the exosome content is directly reflecting their biogenesis and includes tetraspanin (CD9, CD63, CD81) and ESCRT proteins (TSG101, ALIX). Post-translational modifications including ubiquitination and sumoylation were also suggested to contribute to the sorting of specific proteins in exosomes [22-24]. Other exosome components, such as mRNAs and miRNAs, appear to be selectively sorted to exosomes [25]. Several studies have shown that the pool of miRNAs present in exosomes does not merely reflect their cellular abundance [26, 27]. Some miRNAs seem to be preferentially directed to exosomes whereas others appear to be retained in parental cells [28]. Moreover, the exosomal miRNA cargo can be modified in response to environmental stressors, suggesting an active sorting mechanism.

Different pathways, RNA motifs and molecules have been reported to contribute to the sorting of miRNAs in exosomes. Among them, miRNA target genes have been suggested to influence the loading of these small non-coding RNAs inside the vesicles. For example in macrophages, an inverse correlation was observed between the amount of miRNAs released in exosomes and the abundance of their target transcripts present in the cells [29]. Changes in the balance between miRNAs and their targets influence the shuttling of the non-coding RNAs from the cytoplasm/P-bodies, where the miRNAs are active, and the MVBs, where exosomes are formed [29]. In glioblastoma cells, mRNAs enriched in exosomes were found to include in their 3'UTR a "zipcode" sequence of 25 nucleotides, containing a CUGCC core and a miRNA-binding site for miR-1289. Therefore, a dynamic mRNA/miRNA mechanism may exist to tag genomic material to exosomes [30].

Some miRNAs bearing a specific sequence were also found to be enriched in exosomes. Villarroya-Beltri *et al*. analyzed the sequences of miRNAs accumulating in exosomes from T lymphocytes and observed an enrichment of the GGAG motif [23]. The authors found that sumoylation of the ribonucleoprotein hnRNPA2B1 favors it's binding to miRNAs containing the GGAG motif and promotes their sorting into exosomes. Santangelo et al. identified another motif (GGCU) in the 3' region of miRNAs present in hepatocyte exosomes [31]. This sequence was found to permit the interaction with the RNA-binding protein SYNCRIP that is part of the exosomal machinery. Downregulation of SYNCRIP led to an impairment in the sorting of miRNAs to exosomes. Another mechanism affecting the accumulation of miRNAs in exosomes relies on the addition of a nucleotide at the 3'end of the non-coding RNA. Indeed, Koppers-Lalilc and colleagues observed in B cell lymphoma cell lines that the isoforms of miRNAs adenylated at the

3'end are mainly retained in the cells whereas those that are uridylated are enriched in exosomes [32].

Proteins important for miRNA-mediated translational repression, such as Ago2 and GW182, are also present in exosomes [33]. In colon cancer cell lines, activation of the KRAS-MEK-ERK signaling pathway, but not of AKT, inhibits the shuttling of Ago2 in exosomes and concomitantly reduces the amount of Ago2-dependant miRNAs in extracellular vesicles [34]. Interestingly, phosphorylation of Ago2 decreased its interaction with multivesicular endosomes and reduced the secretion of this protein inside exosomes. These data suggest that proteins interacting with miRNAs may influence their transfer inside exosomes.

Exosome uptake

After their release in the extracellular space, exosomes can diffuse in the bloodstream and in other body fluids. The exosome cargo is protected from degradation and is rapidly taken up by different organs, such as liver, lung and spleen. The half-life of circulating exosomes has been evaluated to be around 2 minutes for labelled exosomes injected intravenously in mice but some exosomes were still detectable in the circulation hours after injection [35, 36]. It is still unclear how, or even if, exosomes are specifically targeted to recipient cells and how they can deliver their cargo inside the cells. However, the mechanisms governing the entry of exosomes in recipient cells are beginning to be unveiled. So far, three main routes have been described (Figure 1): 1) direct interaction of the exosome component with a membrane receptor [37, 38], 2) fusion of the exosomes with the plasma membrane of the receiving cell [39] and 3) internalization of the exosomes by the recipient cells by phagocytosis, or by clathrin-, caveolin-mediated endocytosis [40]. Tian and colleagues performed real-time fluorescence microscopy and single particle tracking to follow the internalization of exosomes by PC12 cells [41]. After 30 minutes of incubation, they

observed that the labelled exosomes were slowly moving at the cell surface in a drifted mode, probably as a result of the lateral diffusion of the receptors bound to the exosomes. Few hours later, a large number exosomes were internalized and most of them were trapped in endocytic-derived vesicles, suggesting that in PC12 cells most of them enter by endocytosis. Similar findings were reported by Heusermann et al. [42]. Using single-vesicle dye tracing, the majority of the exosomes from HEK293 cells were found to enter recipient cells as intact vesicles primarily via filopodia, to be sorted in endocytic vesicles and to scan the endoplasmic reticulum before being targeted to lysosomes. A small fraction (10 to 20%) of the labelled exosomes were found to remain at the plasma membrane. Finally, fusion of labelled exosomes with plasma membrane was observed in melanoma and dendritic cells using spectrofluorometry and confocal or fluorescence time-lapse microscopy [27, 39].

As discussed for the secretory mechanisms, the mechanism of uptake may differ between heterogeneous populations of exosomes or between different cell types. Further research is needed to better understand the mechanisms involved in the uptake of exosomes and the delivery of their cargoes inside the recipient cells.

Transfer of nucleic acids between cells mediated by exosomes

Pioneer studies have demonstrated the shuttling of bioactive mRNAs and miRNAs between cells *in vitro* [5, 6]. The transfer of genetic material by exosomes has now been elegantly confirmed also *in vivo* using the Cre-Lox system. This approach is based on the capacity of exosomes to transfer the Cre mRNA from Cre-producing cells to receiving cells containing a floxed gene, resulting in the expression of a specific marker. Ridder et al. used transgenic mice expressing the Cre recombinase specifically in the hematopoietic lineage [43]. They found that the Cre mRNA

accumulates in blood exosomes and can be transferred to cells located in different organs, including liver, lung, intestine and brain where they induce the expression of the floxed gene. Of interest, while the number of recombination events was very low in healthy mice, it increased dramatically in response to inflammatory injuries. Zomer et al. demonstrated that Cre-expressing malignant tumor cells release EVs that induced red-to-green Cre-dependent conversion in recipient benign tumor cells [44]. The transfer of Cre mRNA between tumor cells *in vivo* was observed both locally and systemically. Interestingly, exosomes from malignant tumor cells favored the migratory and metastatic properties in recipient benign tumor cells [44]. Appropriate controls were performed in these two studies to exclude the possibility that the observed recombination events were resulting from an unspecific expression of Cre recombinase in recipient cells, heterotypic cell fusion and/or endogenous misexpression of the floxed gene. Taken together, these studies support a role for exosomes in inter-cellular and inter-organ communication.

Metabolic organs releasing exosomes

Pancreatic β-cells

Pancreatic β-cells play a central role in the regulation of glucose homeostasis by secreting insulin, the main hormone controlling the uptake and the utilization of this carbohydrate. The amount of insulin released by β-cells is continuously adjusted to the concentration of glucose, but also of other nutrients, such as amino acids and fatty acids present in the bloodstream to precisely match the organism needs. A number of studies have demonstrated the fundamental role of miRNAs in β-cell development, maturation, function and survival. Deregulation of miRNA expression has been reported in pancreatic islets from diabetes animal models and from human

diabetic donors [45-48]. More recently, miRNAs were found to be transferred between β-cells [26], adding another dimension of complexity in the miRNA-mRNA network. The exosomal miRNA cargo is not simply a mirror image of the content of the parental β-cell. Global profiling of the miRNAs present in exosomes from β-cell lines or from pancreatic islets revealed that a subset of them is preferentially released in exosomes while others are retained in the parental cells [26, 49]. Pathophysiological conditions associated with diabetes affect the composition of the exosomal cargo of β-cells [26, 50]. Indeed, incubation of non-treated recipient β-cells with exosomes released by cytokine-treated MIN6 cells induced cell death that could be prevented by silencing Ago2 in recipient cells. These data suggest that miRNAs are mediating, at least in part, the detrimental effect of exosomes on β-cell survival. Other components of the exosomal cargo could also be involved in this crosstalk between β-cells (Figure 2). Using the insulin-secreting cell line INS-1 as a model, Zhu and colleagues observed that treatment with low concentrations of pro-inflammatory cytokines favors the release of neutral ceramidase (NCDase) in exosomes, resulting in the production of sphingosine 1-phosphate (S1P) and in the activation of the S1P receptor 2 in recipient β-cells. Incubation with NCDase-containing exosomes protects the recipient β-cells against apoptosis induced by high concentrations of cytokines or palmitate [51, 52]. However, pro-inflammatory cytokines have dual effects on β-cells. At high concentration, cytokines induce apoptosis whereas low concentrations of these inflammatory mediators favors the function and the survival of β-cells. Interestingly, these dual effects were also observed in recipient cells incubated with exosomes released by β-cells incubated at low or high doses of cytokines, which promoted cell survival or apoptosis, respectively [26, 52]. Of course, more studies will be required to confirm this hypothesis, but the data obtained so far suggest that exosomes contributes to achieve a concerted response of β-cells to inflammatory stimuli.

Long non-coding RNAs (lncRNAs) are emerging as important players in the regulation of β-cell function [53, 54]. Some of these non-coding transcripts may potentially also be released in exosomes. In a previous study, we identified a group of lncRNAs that is upregulated in the islets of pre-diabetic NOD mice [55]. Some of them, lncRNA-1 (Gm5970), lncRNA-2 (Al45BIS) and lncRNA-3 (BC002288) are detectable also in exosomes of MIN6 cells (Guay, Menoud and Regazzi, unpublished observations). While incubation of MIN6 cells with pro-inflammatory cytokines promoted the expression of these lncRNAs inside the cells, the levels of lncRNA-2 and lncRNA-3 released in exosomes were not affected by this treatment. In contrast, the level of lncRNA-1 was found to increase in exosomes of MIN6 cells incubated for 48h with cytokines (Guay, Menoud and Regazzi, unpublished observations). These data suggest that lncRNAs and potentially other non-coding RNAs can be secreted in exosomes and eventually transferred to recipient cells.

Beside non-coding RNAs, exosomes released by β-cells can also carry mRNAs and proteins. Proteomics analysis have been performed on exosomes released by β-cell lines. Lee *et al.* [56] identified 270 proteins in exosomes released by NIT-1 insulinoma cells, including metabolic proteins, endocytosis/exocytosis related proteins, chaperones, cytoskeletal proteins, membrane transporters/ion channels, signaling molecules and nucleic acid binding proteins. Palmisano and colleagues [50] focused on membrane-associated proteins released in exosomes from NHI 6F Tu28 cells and identified 191 proteins including 38 glycosylated and 15 phosphorylated proteins. Interestingly, as observed for non-coding RNAs, the protein composition of exosomes was changed in response to pro-inflammatory cytokines [50]. It is now well accepted that insulin-secreting cell lines and primary islets of mice, rats and humans release exosomes [26, 49, 50, 57], but the relevance of this process for inter-organ crosstalk is just beginning to emerge. Growing evidence

suggests that exosomes participate in the dialogue between immune cells and β-cells during type 1 diabetes development (T1D). T1D is an autoimmune disease characterized by infiltration of pancreatic islets by immune cells, leading to selective elimination of the β-cells. Exosomes isolated from culture media of insulinoma or of islet cells promote the immune response by activating antigen-presenting cells (APC), autoreactive marginal zone-like B cells and T lymphocytes [57-60]. Interestingly, intravenous injection of β-cell exosomes in non-obese diabetes resistant NOR mice accelerated the insulitis development [60]. Exosomes were also found to be secreted by mesenchymal stem cell (MSC)-like cells present in the islets of NOD mice. These exosomes are highly immunogenic and can activate both autoreactive B and T cells. When injected intravenously, MSC-like cell exosomes favored immune infiltration and β-cell destruction of NOD mice [61]. The exact mechanisms by which insulinoma exosomes activate the immune cells remains to be uncovered. However, exosomes from rat and human islets were found to carry GAD65, IA-2, and proinsulin autoantigens and also immunostimulatory chaperones that can favor the stimulation of APC and the activation of T-cells [57].

The cross-talk between β-cells and endothelial cells is of particular importance in the context of islet transplantation for the treatment of T1D patients. Despite important progress in the transplantation methods over the past decade, most of the patients receiving the islets fail to remain insulin-independent in the long-term [62]. The days immediately following the transplantation are crucial for islet vascularization, survival and function. Figliolini and colleagues [49] investigated if exosomes released by human islets are able to promote the migration and proliferation of islet endothelial cells (IEC) and, therefore, to favor islet vascularization and engraftment. Exosomes isolated from human islets were found to carry a subset of miRNAs called angiomiRs (miR-27b, miR-126, miR-130 and miR-296) and some mRNAs of genes associated with angiogenesis (eNOS

and VEGFa). Incubation of IEC with islet exosomes increased the migration and proliferation of recipient endothelial cells and protected them from apoptosis. Moreover, islet exosomes were observed to induce the expression of pro-angiogenic (angiopoietin1, VEGFa, VEGFR1 and VEGFR2) and anti-apoptotic factors (BCL-2) in IEC and to repress anti-angiogenic (thrombospondin1) and pro-apoptotic (BAD) molecules [49]. The dialogue between islet and endothelial cells was also investigated in the reverse direction. Cantaluppi *at al.* explored the effects of EVs isolated from the supernatant of human endothelial progenitor cells (EPC) on islet graft function and vascularization [63]. Incubation of human islets with EPC-exosomes favored insulin release and β-cell survival, induced islet endothelial cell proliferation and migration, and the formation of capillary-like structures. *In vivo*, addition of EPC-exosomes to human islets within subcutaneously xenotransplantated in SCID mice was found to enhance their vascularization. The transfer of exosomal miRNAs may participate in this vascularization process. In fact, exosomes from EPC lacking Dicer, the enzyme required for miRNA biogenesis, carry less miR-126 and miR-296 and exert reduced proliferative and angiogenic effects on IEC (Figure 2).

Skeletal muscles

Skeletal muscles account for about 40% of the body mass and are the predominant site for insulin-dependent glucose uptake. Moreover, the insulin-resistant state of skeletal muscles is considered the primary defect leading type 2 diabetes development [64]. Muscles secrete a large number of proteins and cytokine signaling molecules like myokines known to be important in the regulation of nutrient homeostasis [65]. Recently, skeletal muscles were found to secrete also exosomes containing proteins and miRNAs that can be transferred to adjacent muscle cells [66-69]. Moreover, exosomes secreted by muscle cells exposed to fatty acids induced the expression of genes involved in cell cycle and in muscle differentiation of recipient myotubes and favored

myoblast proliferation [66]. There is emerging evidence indicating that muscle exosomes may also be part of the inter-organ network governing glucose homeostasis. Intravenous or intramuscular injection of mice with labelled-exosomes from muscle cells led to their accumulation in various metabolic tissues including pancreas, liver, gastro-intestinal tract and distal muscles [66, 70]. *In vitro*, exosomes from muscle cells were taken up by the β-cell line MIN6 and resulted in an increase in the level of the muscle-specific miR-206, confirming the possible crosstalk between insulin-sensitive and insulin-releasing cells [70]. The authors next investigated if exosomes released by skeletal muscles under insulin-resistant state can affect the functions of β-cells. For this purpose, prior to skeletal muscle isolation and exosome collection, the mice were fed either a standard chow diet or a lipid-enriched diet for 16 weeks. Interestingly, incubation of MIN6 cells with muscle exosomes from mice fed with the lipid-rich diet resulted in an increased proliferation of the insulin-secreting cells. This was attributed to the horizontal transfer of miR-16 and the consequent downregulation of its target gene *Ptch1*. The transfer of miRNAs from muscle exosomes to β-cells may account, at least in part, for the increased size of the islets observed in mice fed on a lipid-rich diet to compensate for the insulin resistant state [70] (Figure 2). More experiments will be needed to precisely understand the mechanisms governing the exosome-mediated crosstalk between skeletal muscles and β-cells under physiological and pathological conditions.

In addition to their contribution to whole-body metabolism and energy homeostasis, skeletal muscles are of course necessary for body locomotion. Endurance exercise produces multisystemic benefits on human health and protects against obesity and diabetes development. Interestingly, the amount of circulating exosomes, and also circulating miRNAs, was found to be increased in an intensity-dependent manner in response to endurance exercise. Exosomes released by skeletal muscles, but also from other tissues, may potentially participate to the autocrine and

paracrine signals leading to the systemic benefits of exercise [71]. However, the precise mechanisms through which they could exert such effects remain to be uncovered.

Adipose tissue

The adipose tissue is a major player in energy homeostasis, but was for a long time considered only as an inert organ responsible for lipid storage. We now known that the adipose tissue regulates whole-body metabolism and releases several hormones and cytokines called adipokines. Under inflammatory condition associated with obesity, the adipose tissue releases the adipokines TNFα, IL-6 and retinol-binding protein-4 (RBP-4) that favor the development of systemic insulin resistance [72]. Exosomes may represent additional vehicles for the communication between the adipose tissue and other metabolic organs to regulate blood glucose homeostasis. Adipocytes and adipose stromal cells have been reported to release exosomes in their culture media [73]. Dheng and colleagues observed that the adipose tissue of mice fed a high fat diet or of *ob/ob* mice release more exosomes than the adipose tissue from control lean animals [74]. When injected in control mice, labelled-exosomes from the adipose tissue of *ob/ob* mice were taken up by monocytes favoring their differentiation into macrophages, the production of the IL-6 and TNFα and the development of an insulin resistance state. These effects were not observed in mice injected with exosomes from adipose tissue of lean animals. The exosomes released by the adipose tissue of *ob/ob* mice were found to contain higher levels of the RBP4 protein, to activate the TLR4/NFκB pathway and to promote the release of IL-6 and TNFα by macrophages (Figure 2). Finally, exposure of myocytes to conditioned media of macrophages incubated with exosomes from *ob/ob* adipocytes resulted in a lowering of AKT phosphorylation in response to insulin and reduced basal and insulin-stimulated glucose transport. Similar results were obtained with

exosomes isolated from human subcutaneous and omental adipose tissue cultured *ex vivo* [75]. Incubation of human adipocytes with conditioned media of macrophages treated with adipose tissue-exosomes led to a reduction of AKT phosphorylation in response to insulin. Exosomes from adipose tissue were also found to interfere with the insulin signaling in liver and muscle cells [75, 76]. Finally, a correlation was observed between the number of exosomes released by omental adipose tissue and the HOMA insulin resistance index. Taken together these findings suggest that under obesity conditions the release of exosomes by the adipose tissue promotes the inflammatory state associated with the development of insulin resistance.

In a recent paper, Thomou et al. investigated if the miRNAs released in exosomes produced by different types of fat can regulate gene expression in distant metabolic organs [77]. They first observed a strong reduction in the level of serum miRNAs in mice lacking the miRNA-processing enzyme Dicer specifically in adipocytes (ADicerKO). A similar reduction in circulating exosomal miRNAs was measured in human patients with congenital or HIV-associated lipodystrophy. These patients suffer from a generalized loss of adipose tissue or have a marked reduction in the level of Dicer in fat, respectively. Interestingly, a set of 30 miRNAs were found to be decreased in the two human cohorts and in ADicerKO mice. The level of circulating exosomal miRNAs could be restored, to about 50% of the normal levels, by transplanting different fat depot from wild type mice. Interestingly, the impaired glucose tolerance observed in ADicerKO mice was significantly improved by transplantation of brown, but not white, adipose tissue, with a trend to improve also insulin sensitivity. Looking at the mechanism potentially linking the release of miRNAs from brown adipose tissue to insulin resistance, the authors demonstrated both *in vitro* and *in vivo* that at least part of the effect was mediated by the transfer of miR-99b from the adipose tissue to the liver and the consequent inhibition of Fibroblast Growth Factor 21 (FGF21) expression (Figure 2).

As a proof-of-concept and to further substantiate the possibility of a crosstalk between the two tissues, the authors used two cohorts of mice. In a first cohort, the mice were engineered to express hsa-miR-302f in brown adipose tissue, whereas in the second cohort, the mice were expressing a 3'UTR-hsa-miR302f reporter in the liver. When exosomes isolated from the first cohort of mice were injected in the second cohort, the luciferase activity of the reporter was massively downregulated, demonstrating that exosomes from adipose tissue can indeed transfer bioactive miRNAs to the liver.

The miRNAs released by the adipose tissue might also affect other metabolic organs or favor the occurrence of complications related to diabetes. Circulating levels of miR-130b were found to be increased in serum of obese mice and humans and to positively correlate with the body mass index. Mechanistically, increased levels of TGF-β in obese mice favor the release of miR-130b by adipocytes. Incubation with adipocyte conditioned media led to a rise of miR-130b in C2C12 myotubes where it affected the expression of target genes such as PGC-1α. The role of exosomes was not directly investigated in this study, but inhibition of neutral sphingomyelinase 2, an enzyme involved in exosome secretion, reduced the release of miR-130b by adipocytes [78]. In another study, Fang et al. investigated the mechanism linking rosiglitazone, an insulin-sensitizing agent and PPARγ agonist, to cardiac hypertrophy [79]. They observed that *in vivo* ablation of PPARγ in adipocytes prevents cardiac hypertrophy elicited by rosiglitazone. They further discovered that activation of PPARγ signaling in adipocytes increases the expression and secretion of miR-200a that is then transferred via exosomes to cardiomyocytes. Following its transfer, miR-200a blocks TCS1 expression, promotes mTOR phosphorylation and favors cardiomyocyte hypertrophy.

Liver

Exosomes released by hepatocytes and different liver cell lines have been suggested to impact on diverse liver functions and to be involved in liver diseases [80, 81]. The increased prevalence of obesity and metabolic syndrome favors the development of liver steatosis that can progress to non-alcoholic fatty liver disease (NAFLD) and in nonalcoholic steatohepatitis (NASH). Hirosva and colleagues investigated whether exosomes released by hepatocytes may be part of the mechanisms that activate the macrophages and promote inflammation in liver diseases [82]. They observed that mouse, rat and human hepatocytes release increased amounts of microvesicles in response to the saturated fatty acid palmitate, but not following incubation with the unsaturated fatty acid oleate. Inhibition of two component of exosome biogenesis and secretion, neutral sphingomyelinase and Rab27, did not prevent this effect in Huh7 hepatocyte cells suggesting that the secreted vesicles are not exosomes. Nonetheless, lipotoxic conditions induced the released of the protein TRAIL in hepatocyte microvesicles that favored the activation of macrophages and the release of pro-inflammatory cytokines in a RIP1 and NFκB-dependent manner. An increase in liver microvesicles was also observed in serum of mice fed with a diet containing high amounts of saturated fat, fructose, and cholesterol. Interestingly, blockade of microvesicle release for 2 weeks obtained by injecting mice with fasudil decreased macrophage activation and hepatocyte inflammation of mice fed with this diet. This study highlight a possible role for microvesicles in NASH disease [82]. However, other investigations are needed to determine if exosomes play also a role in liver disorders associated with metabolic syndrome and if the cargo of liver exosomes can also be transferred to other organs.

Perspectives

The capacity of exosomes to transfer proteins and miRNAs to distantly located cells opens the possibility of their utilization as novel drug vehicles. RNA interference (RNAi)-based therapy is using small interfering RNA (siRNA) or miRNAs to specifically silence a gene of interest. This strategy is very promising but so far only few clinical trials have been performed. Conventional routes of oligonucleotide delivery have to face rapid degradation, poor bioavailability, unspecific organ targeting and the inability to cross the blood-brain barrier [83]. The utilization of exosome-like vesicles as vehicles for the transport of siRNA/miRNA may open the way to new strategies to specifically and efficiently deliver them to the target cells.

Exosomal carriers provide advantages of both cell-based drug delivery and nanotechnology. They can travel from one cell type to another, they deliver their content across the plasma membrane in active form and they can pass the blood-brain barrier [84]. Two different approaches have been described for oligonucleotide loading into exosomes. The first, developed by Alvarez-Erviti and colleagues, uses electroporation for incorporating siRNAs in dendritic cell-derived exosomes [85]. A similar strategy can be applied to load up to 3000 miRNA molecules in exosomes [86]. However, the efficiency of exosome electroporation varies according to the experimental conditions [84]. The second approach, takes advantage of the release by parental cells of part of their cellular components in exosomes. Indeed, the fusion of an antigen with a protein known to be targeted to exosomes was shown to result in its loading in the vesicles and its extracellular release [87]. An analogous strategy could potentially be envisaged to tag and load the miRNAs of interest in exosomes.

Few studies have already demonstrated the efficiency of exosome-mediated delivery of siRNAs to recipient cells [84]. For example, exosomal delivery of the antimiR-150 in mice

neutralized miR-150, diminished VEGF levels and attenuated angiogenesis [88]. Finally, Alvarez-Erviti *et al*. reported that genetic modifications of parental cells could be used to target exosomes to the disease site [85]. They succeeded in engineering exosomes containing a siRNA directed against GADPH and the neuron-specific peptide RVG. When injected in mice, a specific gene knockdown was observed in neurons, microglia and oligodendrocytes, demonstrating the therapeutic potential of exosomes-mediated siRNA delivery.

CONCLUSION

The number of studies supporting a role for exosomes in metabolic organ cross-talk is rapidly growing. Several aspects of this new communication mode remain however to be elucidated. For example, it is still unclear how miRNAs, mRNAs and proteins are selectively loaded in exosomes and how exosomes can be targeted to specific cell types. Moreover, we still don't know whether a given cell can secrete subpopulations of exosomes with different cargo composition and function. Additional studies will be needed to determine the potential and the limitations of exosomes and their miRNA cargo in intercellular communication but they promise to open an entirely new perspective in our understanding of inter-organ crosstalk.

CALLOUTS (25-40 words each, 3-5)

1) Exosomes are small extracellular vesicles that transport proteins and nucleic acids, which can be transferred in active form to nearby cells or to distant organs.
2) miRNAs are important regulators of gene expression by. acting inside the cells producing them but also in distant cells after their transfer by exosomes.

3) Islet exosomes can act in an? autocrine manner to favor a concerted response of the β-cells, or in paracrine manner to communicate with surrounding endothelial or immune cells.
4) In a mouse model of diet-induced obesity, skeletal muscles were found to release in exosomes miRNAs capable of promoting β-cell proliferation to compensate for insulin resistance.
5) miRNAs, including miR-99b, released by the brown adipose tissue are transported to the liver by exosomes where they can inhibit their target genes.

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FIGURE LEGENDS

Figure 1: Schematic model of exosomal miRNA transfer. miRNAs produced by donor cells can be sorted to exosomes during multivesicular bodies (MVB) formation (1). Exosomes are released in the extracellular space upon fusion of the MVB with the plasma membrane (2). The exosomal miRNAs can then be delivered to recipient cells by endocytosis (3) or by fusion of the exosomes with the plasma membrane (4). Exosomes may also bind to a receptor and activate specific signaling pathways (5).

Figure 2: Schematic representation of the involvement of exosomes in inter-organ cross-talk. Insulin-secreting β-cells and insulin-sensitive tissues release in exosomes containing proteins and miRNAs that can be transferred to other metabolic organs, or to immune or endothelial cells. Exosomes are acting in both autocrine and paracrine manners to favor the maintenance of glucose homeostasis or to aggravate insulin resistance.

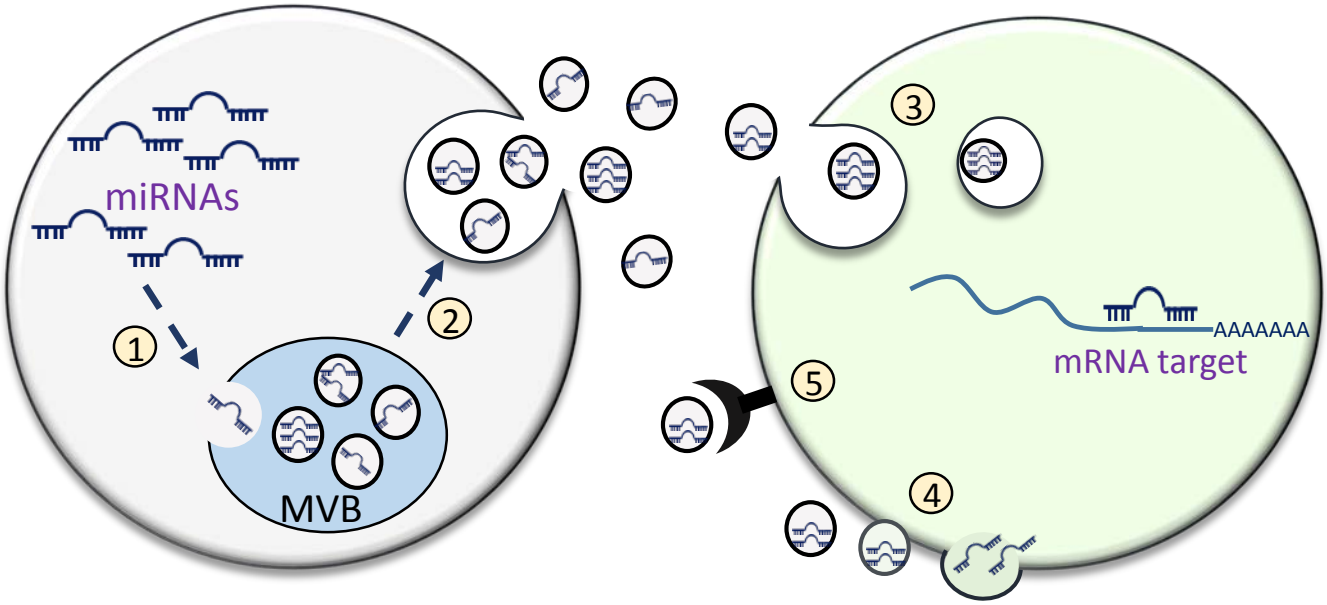

Figure 1 Guay and Regazzi

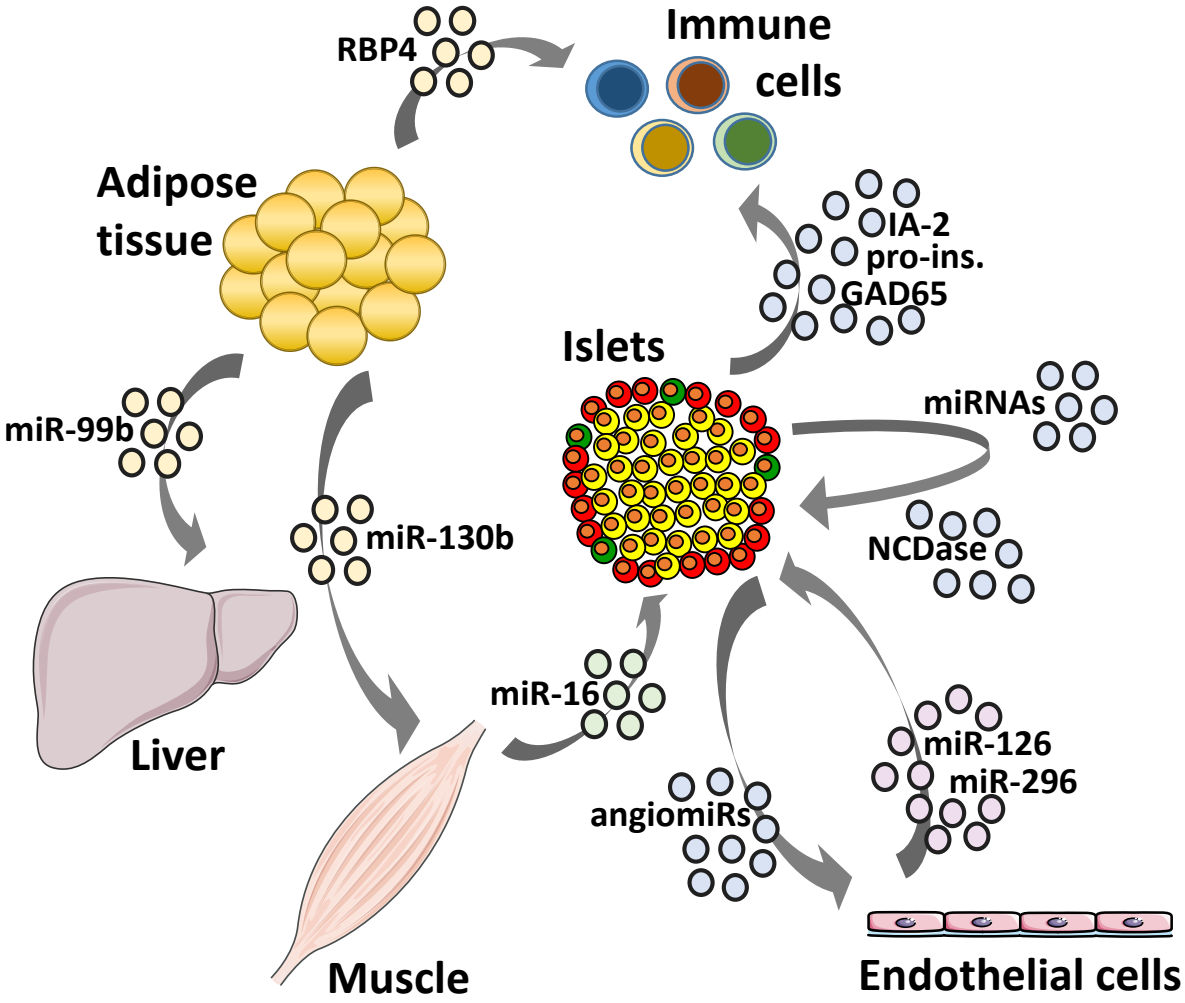

Figure 2 Guay et al.