Editorial

Pancreatic cancer-derived exosomes: new role in paraneoplastic syndromes?

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Recent studies show that cells can communicate through the exchange of bioactive molecules via microvesicles. These small vesicles include ectosomes (100–1,000 nm) and exosomes (30–100 nm), which are shed or secreted by almost all cell types (1). Endosome-derived microvesicles, known as exosomes, are secreted as part of multivesicular bodies into the extracellular space. Secreted microvesicles can be engulfed by tissues locally or carried in biological fluids to distant sites in the body (2). Intercellular communication can be initiated through interactions between circulating microvesicles and transmembrane proteins of target cells. Microvesicles fuse with target cell membranes, transferring cell surface molecules and receptors from donor to recipient cells. In addition, target cells engulf exosomes through endocytosis, resulting in the intracellular release of bioactive molecules, which include proteins, lipids, microRNAs, mRNAs, DNA, and metabolites (3-6). Tumor exosomes have been shown to be involved in various biological functions such as promoting angiogenesis and inducing apoptosis of activated cytotoxic T cells to create an immunosuppressive microenvironment that promotes cancer progression (7-9).

Pancreatic cancer is one of the most lethal cancers with an overall 5-year survival rate about 5%. The poor prognosis of the disease is mainly due to the difficulties in early stage detection, high metastatic potential and resistance to conventional therapies (10). Pancreatic cancer-induced paraneoplastic diabetes often associated with profound weight loss, unlike type 2 diabetes, appears up to 2 to 3 years before the diagnosis of pancreatic cancer. Thus, the presence of weight loss might be an important indicator of the development of pancreatic cancer-induced diabetes and pancreatic cancer before diagnosis. In the absence of symptoms of muscle loss, which is a hallmark of cachexia, this paraneoplastic weight loss is likely due to the loss of adipose tissue (11). Increasing number of studies are focusing on delineating the roles of pancreatic cancer-derived exosomes in early detection and intervention of the disease. For examples, pancreatic cancer-derived exosomes were reported to be involved in liver pre-metastatic niche formation in the context of pancreatic cancer metastasis (10) and the dysfunction of dendritic cells that negatively modulates the anti-tumor immune response (12). Circulating pancreatic cancer-derived exosomes were also reported to be used as biological markers for pancreatic cancer (13). In addition, the role of a 52 amino acid bioactive peptide, adrenomedullin, in pancreatic cancer progression was gaining interest. Adrenomedullin is originally isolated from human pheochromocytoma. It is believed to serve as a multifunctional regulatory peptide (14). The expression of adrenomedullin was found to be elevated in pancreatic cancer patients (15). Exogenous adrenomedullin stimulates pancreatic cancer cell proliferation and invasion *in vitro* while overexpression of adrenomedullin promotes cancer growth and metastasis *in vivo*. The effects are abrogated with an adrenomedullin antagonist and adrenomedullin shRNA, respectively (14).

Recently, Sagar and colleagues reported that exosomal transfer of adrenomedullin from pancreatic cancer to adipocytes induces lipolysis via interaction with the adrenomedullin receptors (ADMRs) and activation of p38 and p44/42 MAPKs. The activation subsequently phosphorylates the hormone-sensitive lipase (HSL) to promote lipolysis, which may explain the paraneoplastic phenomena of pancreatic cancer patients including the concomitant weight loss of new-onset diabetes (16). In addition, they showed that

adrenomedullin is contained in pancreatic cancer-derived exosomes and is delivered to adipocytes. The exosomal adrenomedullin then induces lipolysis in adipocytes. They examined lipolysis by accessing glycerol release and the expression of markers for activated lipolysis. They also found that exosomes from patients with pancreatic cancer activate lipolysis in subcutaneous adipocytes compared with exosomes from non-pancreatic cancer control subjects. They further delineated the mechanism that mediates the exosomal adrenomedullin-induced lipolysis and confirmed lack of functionality with the levels of TNF-α, a known lipolytic agent, in pancreatic cancer-derived exosomes with regard to lipolysis. The TNF-α level in pancreatic cancer-derived exosomes is less than 10 pg while lipolysis was only observed at 50 ng of TNF-α. Finally, they also noticed the presence of multiple processed forms of adrenomedullin in exosomes secreted by pancreatic cancer patient-derived cell lines using Western Blot and immunoprecipitation analyses. However, details about possible mechanisms for packaging different forms of adrenomedullin in exosomes were not explored in this study (16).

The same research group previously reported that pancreatic cancer-derived exosomes and the exosomal adrenomedullin cause paraneoplastic β-cell dysfunction, which inhibit insulin secretion through adrenomedullin-induced ER stress and failure of the unfolded protein response (17). Both studies showed that the effects of adrenomedullin are exerted via the interaction with ADMR. They employed the Duolink fluorescence method to access the interaction between adrenomedullin and ADMR. A functional ADMR is a seven transmembrane domain G-protein-coupled receptor containing a calcitonin receptor-like receptor (CRLR) and one of the three specific receptor activity-modifying proteins (RAMPs). CRLR in combination with RAMP2 or RAMP3 produces a functional ADMR. On the other hand, CRLR in combination with RAMP1 produces a receptor for calcitonin gene related peptides (14,16-18).

Although it was reported that, in some cases, binding of exosomes to recipient cells is sufficient to induce physiological changes of the recipient cells, for example, antigen presenting cell-derived exosomes present the MHC-peptide complexes on the surface to antigen-specific T cells. In many other cases, the cargos within the exosomes have to be transferred and delivered into the recipient cells to exert biological changes. It was reported that large extracellular vesicles probably induce phagocytosis whereas smaller sized ones such as exosomes can be internalized by non-phagocytotic process (6). There are various mechanisms of internalization described before depending on the recipient cells including receptor-mediated endocytosis in dendritic cells (19), caveolin-dependent endocytosis in epithelial cells (20) and clathrin-independent but lipid-raft dependent endocytosis in endothelial cells and some cancer cells (6,21). In this study, Sagar and colleagues showed that the interaction between adrenomedullin and ADMR occurs intracellularly, thus they also evaluated the mechanism of the internalization of pancreatic cancer-derived exosomes in adipocytes. They examined exosome uptake by adipocytes after treating the adipocytes with inhibitors of different endocytotic pathways. Only the non-selective macropinocytosis and receptor independent caveolin-mediated endocytosis were revealed to be involved for the internalization of the pancreatic cancer-derived exosomes (16).

While exosomal transfer of adrenomedullin is described in this study, free adrenomedullin in the tumor microenvironment may also play a role in lipolysis. Differences between interaction of the free secretory adrenomedullin with the surface ADMR and interaction of the exosomal adrenomedullin with ADMR after internalization into recipient cells are not investigated in this study. These two processes may trigger different signaling pathways to exert different biological functions. Understanding the different modes of action of adrenomedullin would be useful for developing therapeutic tools of pancreatic cancer. Nevertheless, this study provides insight into early onset paraneoplastic effects of pancreatic cancer. It was reported that subjects with new-onset diabetes are 8 times more likely to be diagnosed with pancreatic cancer than the general population (16) and the survival rate may be worse in patients with new-onset diabetes before pancreatic cancer resection (22). It is crucial to understand the mechanism of the new-onset diabetes and the concomitant weight loss to develop novel biomarkers for early detection of pancreatic cancer before symptoms occur at late stages. It may also provide insight into therapeutic strategy for the paraneoplastic weight loss. Since the roles of adrenomedullin in paraneoplastic diabetes are rather well-studied, it is possible to alter different steps in the route of pancreatic cancer-derived exosomal transfer of adrenomedullin including biogenesis, assembly and release of exosomes to recipient cells to reduce lipolysis and β-cell dysfunction.

This study suggests that strategies targeting the synthesis and secretion of pancreatic cancer-derived

exosomes may be a viable approach to inhibit lipolysis due to exosomal transfer of adrenomedullin from pancreatic cancer to adipocytes. On the other hand, targeted therapy using engineered exosomes to target adrenomedullin up-regulation in adipocytes could be another approach. Different methods are proposed to engineer the content in exosomes or tailor exosomes for specific transfer to recipient cells. Electroporation of exosome suspension or chemical based transfection of exosomes with cargos of choice and incubation of exosomes with cargos of choice under certain conditions were proved to be able to load exosomes (23). Overexpression of microRNAs that target oncogenic molecules delivered by exosomes, in this case, adrenomedullin, is one of the possible ways to engineer the exosomes for therapeutic purposes. MicroRNAs are comparatively stable and they inhibit a gene function by targeting the 3' untranslated region (UTR) of a gene. Recent study showed that miR-879 interacts with the 3' UTR of *ADM*, which encodes adrenomedullin (24). In addition, ADMR antagonist, which is a 22–52 amino acid adrenomedullin peptide, could be transferred to adipocytes or β-cell specifically via exosomes to abrogate the interaction between adrenomedullin and ADMR (16,17). Since the exosomal surface can be modified by incorporating with different targeting peptides, which allow exosomes to deliver cargos to a particular recipient cell type (23), one can envision that bioengineered exosomes can be designed to specifically deliver the ADMR antagonist and adrenomedullin siRNAs to adipocytes and pancreatic cancer cells, respectively, to suppress the effect of adrenomedullin on lypolysis.

In conclusion, Sagar and colleagues furthered their studies in the role of pancreatic cancer-derived exosomal transfer of adrenomedullin in paraneoplastic diabetes and the concomitant weight loss in pancreatic cancer patients in this publication. Future investigation on potential therapeutic strategies via bioengineering of exosomes and the possibility for adrenomedullin as a biomarker to distinguish new-onset diabetes from type 2 diabetes will be important for early detection of pancreatic cancer and the improvement of survival rate in pancreatic cancer patients.

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Footnote

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