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Pancreatic Cancer–Associated Diabetes Is an “Exosomopathy”

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Abstract

Diabetes may be a consequence of pancreatic cancer, preceding cancer diagnosis. The underlying mechanism is the release exosomes delivering adrenomedullin to β -cells, inducing endoplasmic reticulum stress and perturbations in the unfolded protein response, leading to β -cell dysfunction and death. This knowledge could lead to improved diagnostic strategies for pancreatic cancer.

In this issue of *Clinical Cancer Research*, Javeed and colleagues (1) demonstrate that exosomes released by pancreatic cancer cells (PCCs) in culture and in pancreatic ductal adenocarcinoma (PDAC) patients carry adrenomedullin (AM) which is delivered to the β -cell, inducing endoplasmic reticulum (ER) stress, a failure of the unfolded protein response (UPR), increased β -cell dysfunction and death, and PDAC-associated diabetes mellitus.

Most cases of diabetes mellitus are classified as type I or type II diabetes. The former, termed T1DM is associated with undetectable insulin levels due to autoimmune-mediated destruction of the β -cells and a propensity to develop ketoacidosis. The latter, termed T2DM is associated with insulin resistance, elevated, normal, or slightly decreased insulin levels, a failure of the beta cell to overcome insulin resistance, and an inability of the liver to suppress inappropriate hepatic glucose release. Among the population of diabetic patients in the U.S. it is estimated that ~5% have T1DM and ~90% have T2DM. Importantly, the Centers for Disease Control and Prevention (CDC) estimates that approximately 29 million people in the U.S. had diabetes mellitus in 2012 and that in 8 million of these individuals diabetes had not yet been diagnosed (2). Longstanding T2DM is a risk factor for PDAC (3). Additional risk factors include inherited familial disorders, tobacco smoking, obesity, chronic pancreatitis, heavy alcohol intake, and diets rich in saturated fats and low in fruits and vegetables (3). However, the relationship between DM and PDAC is complex, and loss of glycemic control and diabetes mellitus can occur as a consequence of PDAC and can precede the diagnosis of PDAC by a few weeks, to a few months, to 2-3 years (4). This type of diabetes, termed pancreatogenic diabetes, or type 3c diabetes (T3cDM), is much less common than either T1DM or T2DM. The underlying etiology in most cases is chronic pancreatitis (CP), and it has been estimated that CP and PDAC contribute to approximately

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80% and 8% of T3cDM, respectively, whereas the remaining cases occur as the result of other types of pancreatic exocrine pathologies, such as pancreatic trauma and cystic fibrosis (5-6).

In this issue of *Clinical Cancer Research*, Javeed and colleagues (1) made the point that PDAC-associated T3cDM, termed PC-DM, is associated with insulin resistance and increased peripheral insulin levels, whereas T3cDM observed in CP is associated with decreased insulin levels due to the loss of insulin-secreting β -cells. Having previously shown that PCC-derived AM inhibits insulin secretion by the β -cell, they now focused on how AM targets the β -cell. They first demonstrated that PCC lines preferentially release exosomes over other forms of extracellular vesicles, and that exosomes were present in both the portal and peripheral venous blood of PDAC patients. They next determined that exosomes derived from PANC-1 PCCs can be internalized by β -cells within 48 hours following their co-incubation. Given that exosomes are of endosomal origin and that endosomes express tumor susceptibility gene 101 (Tsg101), they next confirmed that the exosomes they were isolating express Tsg101. Validation of their pancreatic cancer origin was demonstrated by showing that these exosomes expressed the PDAC-associated protein CA19-9. PCC derived exosomes were then shown to inhibit insulin secretion in human islets and in INS-1 rat insulinoma cells. Importantly, AM receptor blockade abrogated the inhibitory effect of exosomes on insulin secretion, whereas medium depleted of exosomes by ultracentrifugation had no effect on the β -cells.

AM was originally identified as a hypotensive hormone isolated from pheochromocytoma and subsequently shown to exert proliferative and pro-angiogenic effects and to inhibit insulin secretion (7). There are three AM receptors (ADMRs), and all three belong to the 7-transmembrane superfamily of g-protein coupled receptors, but each has a different affinity for AM (8). Among these, the calcitonin receptor-like receptor (CRLR) requires single transmembrane modulating proteins known as receptor activity modifying proteins (RAMPs). It was important, therefore, to investigate the interactions between exosomal AM and ADMR. Accordingly, Javeed and colleagues (1) used a fluorescent-based Duolink assay system in which fluorescent proximity ligation assay probes were attached to antibodies against AM and CRLR. Using this assay, they demonstrated that PCC-derived exosomes enhanced AM/ADMR interactions, and that the addition of the AM 22-52 inhibitor peptide decreased these interactions. They next showed that inhibition of micropinocytosis with amiloride or of caveolin function with Nystatin inhibited exosome internalization. Taken together, these observations confirm that AM/ADMR interactions occur inside the β -cell, and suggest that exosomes-mediated delivery of AM into the β -cell is mediated via both micropinocytosis and caveolin-mediated endocytosis (Fig. 1).

To determine why AM interferes with β -cell function even though it upregulates insulin mRNA and cyclic AMP levels, Javeed and colleagues (1) examined the effects of AM on ER stress and the unfolded protein response. They observed that incubation of INS-1 cells with AM peptide for 48 h, followed by glucose stimulation for 4-6 h, led to an increase in *Bip* and *Chop* mRNA levels, indicating that there was enhanced ER stress. This effect was reversed by AM 22-52, confirming that it was mediated by AM. PCC-derived exosomes also induced increased *Bip* and *Chop* mRNA expression in INS-1 cells, while enhancing

apoptosis and Bip-proinsulin interactions, indicating that in addition to enhanced ER stress there was an AM-induced failure of the UPR pathway (Fig. 1).

This study is important for several reasons. First, it underscores the complex connections between diabetes and PDAC. Second, it demonstrates that PDAC is associated with preferential production of exosomes over other extracellular vesicles. Third, it provides novel insights on how exosomes lead to beta-cell dysfunction, raising the possibility that PDAC-associated diabetes is caused, in part, by exosomes and pointing to a novel exosome-based disease mechanism or “exosomopathy”. Thus, PCDM induces both insulin resistance and beta-cell dysfunction. Fourth, the study draws our attention to the loss of glycemic control as a harbinger of developing PDAC, a deadly cancer that is mostly diagnosed at an advanced stage and that may become the second leading cause of cancer death in the US by 2030 (9). Therefore, sensitive and specific screening tests for the early diagnosis of PDAC are urgently needed, and the current findings raise the possibility that exosomes released by PCCs and their cargo could be assayed in the peripheral circulation to detect PDAC at earlier stages than currently possible, monitor cancer recurrence, and assess prognosis and therapeutic responsiveness.

Future studies should delineate whether pancreatic cancer-derived exosomes also cause insulin resistance which is the mechanism implicated in PC-DM, whether loss of β -cell mass in CP is caused by exosomes targeting the endocrine islets, whether exosome-delivered cargo proteins other than AM, or RNAs and microRNAs within the exosomes, contribute to these or other aspects of PDAC pathobiology, whether AM within the tumor microenvironment can damage the β -cells, and whether activation of ER stress by exosomes-delivered AM is associated with a classical upregulation of PERK, a PKR-like ER kinase (10). Given the high prevalence of T2DM in the population and the realization that approximately 86 million adults had pre-diabetes in 2012 in the U.S. (2), it still remains to be determined which new onset diabetic patients should be screened for PDAC. Nonetheless, in combination with clinical evaluations and assays of plasma microRNAs to distinguish between PDAC and CP (11), the current findings could raise new hope for moving in the direction of earlier PDAC diagnosis.

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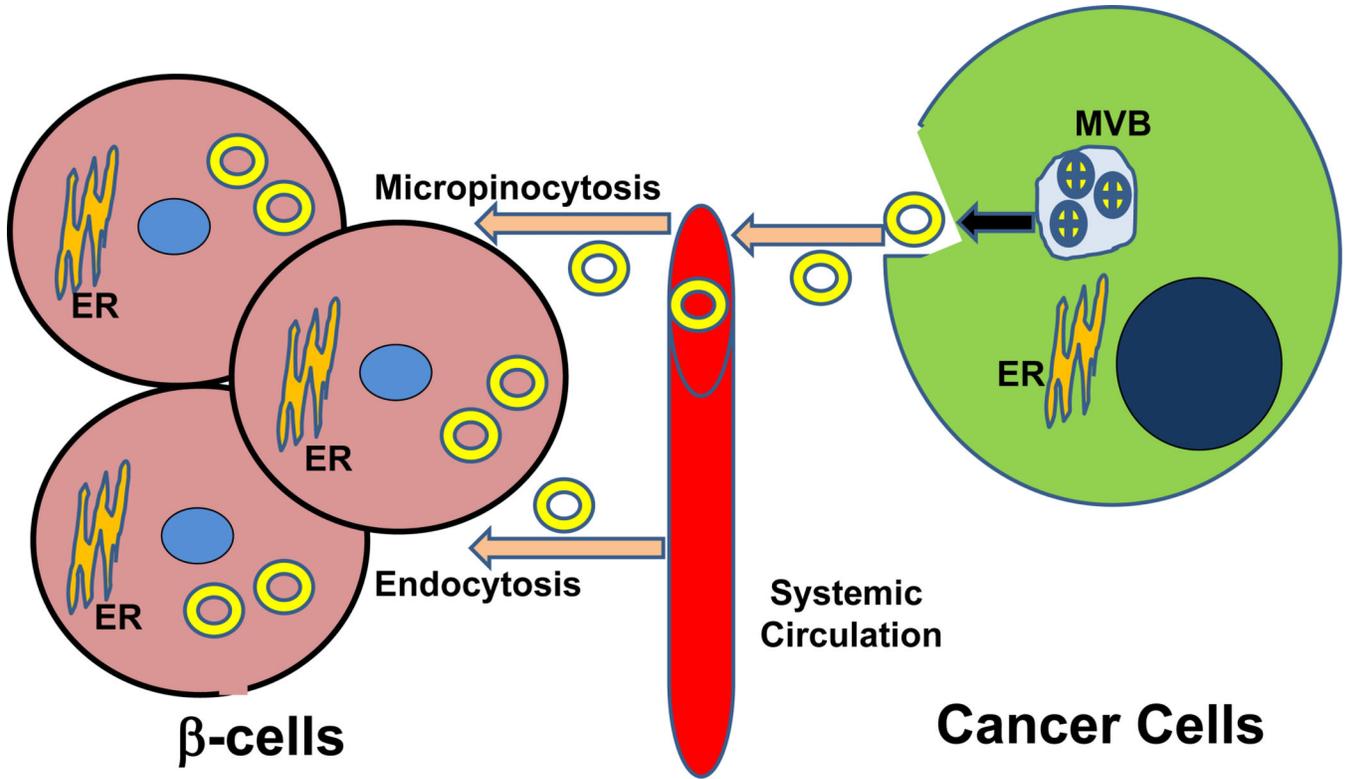


Figure 1. Pancreatic cancer cells express and release adrenomedullin into the circulation as well as into exosomes (shown as small yellow circles) which derive from multivesicular bodies (MVB) which in turn originate from endosomes (not shown). While adrenomedullin released into the circulation does not achieve sufficiently high levels to affect the β-cells, when packaged into exosomes it is delivered into β-cells by endocytosis and micropinocytosis resulting in sufficient quantities of intracellular AM for the induction of endoplasmic reticulum (ER) stress, perturbations in the unfolded protein response, suppression of insulin secretion, and ultimately β-cell death.