Review

Exosomes as Biomarkers and Therapeutic Targets in Pancreatic Cancer

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Abstract: Pancreatic cancer (PC) is a highly malignant tumor with a poor prognosis; indeed, in recent years the incidence and mortality rate have increased. However, the lack of highly sensitive and specific molecular diagnostic markers makes it difficult to classify PC. Exosomes, cell-derived vesicles that contain bioactive molecules, play key roles in cell−cell communication, a process that regulates diverse biological processes in cancer. Here, we highlight recent advances in the roles of exosomes in PC, focusing on their potential utility as diagnostic biomarkers and therapy targets. Although several biomarkers and therapies are being researched, we believe that advances in molecular biology techniques and testing of larger patient cohorts will improve targeted therapy for PC.

Keywords: pancreatic cancer; exosome; biomarker; PGC-1; MIF

Introduction

Pancreatic cancer (PC) is one of the most common malignant tumors of the digestive system, with an incidence rate of 1–2%. Despite improvements in survival rates in different types of cancer over the past 40 years, the overall prospects for PC patients have not improved much: the 5-year survival rate is still only 7% [1]. Major obstacles include difficulty of diagnosis at an early stage (due to a lack of specific biomarkers), metastatic spread when the primary tumor is still too small to detect, and therapeutic resistance due to development of dense fibrous tissue around the tumor [2,3]. Thus, research to identify suitable and specific biomarkers and develop targeted therapies for eligible patient subgroups is of utmost importance. Identification of clinically meaningful approaches relies heavily on molecular biology techniques, the availability of preclinical models, and analysis of larger patient cohorts [4-6].

Almost all cells, including cancer cells, have the potential to release bilayered membrane-bound nanovesicles into the extracellular space and body fluids. Depending on their size, these extracellular vesicles are classified as exosomes, microvesicles, or apoptotic bodies (Table 1). Exosomes, which demonstrate a novel mode of intercellular communication, were first discovered by Trams et al. in the early 1980s [7-9] and are present in nearly all human body fluids, including blood plasma/serum [10], saliva [11], breast milk [12], cerebrospinal fluid [13], urine [14], and semen [15]. Exosomes contain proteins, DNAs, messenger RNAs, microRNAs, and non-coding RNAs, all of which can be transferred to other cells upon fusion, leading to exchange of genetic information and reprogramming of recipient cells. Increasing evidence suggests that tumor cells release large numbers of exosomes, which may affect tumor initiation, growth, progression, metastasis, and drug resistance. Here, we review current studies investigating the effects of tumor-derived exosomes on PC and their potential utility as PC-specific biomarkers.
Table 1. Extracellular vesicles

<table>
<thead>
<tr>
<th>Extracellular Vesicle</th>
<th>Size</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exosomes</td>
<td>30–100 nm</td>
<td>Released from the plasma membrane and act as a novel mode of intercellular communication</td>
<td>[8]</td>
</tr>
<tr>
<td>Microvesicles</td>
<td>100–1000 nm</td>
<td>Derived directly from the plasma membrane via outward budding</td>
<td>[16]</td>
</tr>
<tr>
<td>Apoptotic bodies</td>
<td>1–5 μm</td>
<td>Released from cells undergoing apoptosis or mechanical stress to prevent leakage of potentially toxic cellular contents from dying cells</td>
<td>[17]</td>
</tr>
</tbody>
</table>

1. Role of exosomes in PC

Accumulating evidence suggests that exosomes play important roles in cancer cell growth, metastasis, and drug resistance. In this section, we will discuss the role(s) of exosomes in PC, along with the underlying molecular mechanisms (Table 2).

Table 2. Role of exosomes in PC

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivin</td>
<td>Promotes tumor growth</td>
<td>[18]</td>
</tr>
<tr>
<td>Wnt5b</td>
<td>Promotes pancreatic cancer cell migration and proliferation</td>
<td>[19]</td>
</tr>
<tr>
<td>miR-21/miR-221</td>
<td>Tumor progression</td>
<td>[20]</td>
</tr>
<tr>
<td>MIF</td>
<td>Promotes metastasis</td>
<td>[21]</td>
</tr>
<tr>
<td>CD44v6</td>
<td>Promotes metastasis and progression</td>
<td>[22]</td>
</tr>
<tr>
<td>Snail</td>
<td>Promotes proliferation and drug resistance</td>
<td>[23]</td>
</tr>
<tr>
<td>GIPC</td>
<td>Sensitivity to gemcitabine</td>
<td>[24]</td>
</tr>
</tbody>
</table>

1.1 Tumor growth

Exosomes derived from PC cells can induce tumor growth. For instance, Harada et al. analyzed the mechanism underlying Wnt5b secretion by cancer cells and found that Wnt5b-associated exosomes promote PC cell migration and proliferation in a paracrine manner [19]. In addition, cancer cells take up exosomes that contain survivin, an anti-apoptotic protein, to protect themselves from genotoxic stress-induced cell death [18]. Moreover, a rich desmoplasia is observed in human PC; Ali et al. revealed crosstalk between cancer-associated fibroblasts, stellate cells and PC cells. Upregulation of miR-21/miR-221 expression may endow PC cells with an aggressive phenotype; therefore, targeting these miRNAs could be useful for developing precision therapies that prevent tumor progression and/or treat PC [20].

1.2 Tumor metastasis

Most cancer deaths are caused by metastasis rather than the primary tumor; it is the metastatic tumors that are generally refractory to available therapies [25]. Researchers are still trying to understand the drivers underlying cancer cell migration and the ability of such cells to escape the site of the primary tumor [26]. A role for exosome-mediated signaling in cancer metastasis is also emerging. For example, Costa-Silva et al. demonstrated that MIF-containing exosomes derived from PC cells induce TGF-β production by liver Kupffer cells, which in turn upregulates fibronectin expression by hepatic stellate cells and increases recruitment of bone marrow-derived cells, ultimately leading to formation of a pre-metastatic niche in the liver [21]. Moreover, CD44 variant isoform v6 (CD44v6), which is transferred from cancer-initiating cells (CICs) to non-CICs via exosomes, promotes metastasis. Wang et al. found that knocking down CD44v6 in human PC cell lines led to loss of CIC characteristics and a marked reduction in tumor progression [22].
1.3 Drug-resistant tumors

Resistance to chemotherapy, radiation, and targeted therapies remains a major stumbling block in terms of cancer treatment [27]. The role of exosomes in drug resistance is an emerging area of intense research. Cancer-associated fibroblasts (CAFs) comprise the majority of the PC tumor bulk. Current efforts to eradicate these tumors focus predominantly on targeting the proliferation of rapidly growing cancer epithelial cells. Richards et al. found that CAFs are intrinsically resistant to gemcitabine and release high numbers of exosomes. These exosomes increase expression of Snail, a chemoresistance-inducing factor, by recipient epithelial cells, thereby promoting proliferation and drug resistance [23]. Bhattacharya et al. showed that GAIP-interacting protein C terminus (GIPC) regulates cellular trafficking pathways by modulating the secretion, biogenesis, and molecular composition of exosomes. Knockdown of GIPC led to overexpression the drug resistance gene ABCG2 in exosomes and increased the sensitivity of PC cells to gemcitabine [24].

2 Exosomes as PC biomarkers

PC is one of the most malignant tumors; the high mortality is largely a consequence of diagnosis at an advanced stage when the tumor can no longer be surgically resected. PC lacks symptoms during tumorigenesis, progression, and metastasis [9,28]. CA 19-9 is the only diagnostic biomarker reported by The National Comprehensive Cancer Network and is used for screening, determining resectability, and as a prognostic marker after resection [29]. Thus, a huge amount of effort has been devoted to the utilization of exosomes as biomarkers for cancer [30,31]. Some of the promising biomarkers identified in clinical studies of patient body fluids are summarized in Table 3.

<table>
<thead>
<tr>
<th>Material</th>
<th>Biomarkers</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRNA</td>
<td>miR-17-5p</td>
<td>diagnostic</td>
<td>[32]</td>
</tr>
<tr>
<td>DNA</td>
<td>KRAS</td>
<td>diagnostic/prognostic</td>
<td>[33]</td>
</tr>
<tr>
<td>mRNA</td>
<td>EGFR</td>
<td>diagnostic</td>
<td>[34]</td>
</tr>
<tr>
<td>miRNA</td>
<td>miR-1246, 4644, 3976, 4306</td>
<td>diagnostic</td>
<td>[35]</td>
</tr>
<tr>
<td>mRNA</td>
<td>GPC1</td>
<td>diagnostic</td>
<td>[36]</td>
</tr>
<tr>
<td>miRNA</td>
<td>mir-10b</td>
<td>diagnostic</td>
<td>[37]</td>
</tr>
<tr>
<td>miRNA</td>
<td>miR-18a</td>
<td>diagnostic</td>
<td>[38]</td>
</tr>
<tr>
<td>mRNA</td>
<td>MIF</td>
<td>diagnostic</td>
<td>[21]</td>
</tr>
<tr>
<td>mRNA</td>
<td>BRCA1/2</td>
<td>diagnostic</td>
<td>[39]</td>
</tr>
<tr>
<td>Peptide</td>
<td>Adrenomedullin</td>
<td>diagnostic</td>
<td>[40]</td>
</tr>
</tbody>
</table>

2.1 Exosomes as biomarkers for early detection of PC

GPC1

Melo et al. performed a proteomics analysis and identified a new, exciting, and highly sensitive method for the specific diagnosis of PC using exosomes carrying the protein glypican-1 (GPC1). They reported that GPC1 is enriched in exosomes derived from PC cells. The amount of GPC1+ exosomes in 190 PC patients was significantly higher than in those from healthy controls. Furthermore, the amount of GPC1+ exosomes was related with the survival of tumor patients both before and after surgery. Hence, GPC1+ exosomes are a potential biomarker for early screening and prognosis prediction for PC patients [36]. However, some problems remain; for example, the method is not practical at present due to the difficulty of isolating GPC1+ exosomes by flow cytometry.

KRAS
KRAS is a Kirsten ras oncogene homolog belonging to the mammalian Ras gene family and is located at the inner surface of the cell-membrane [41]. The most common mutation site is KRAS G12D [42]. There is much evidence that KRAS mutation is required for pancreatic tumorigenesis; it is also found in early lesions [43]. Christoph et al. extracted DNA from exosomes derived from PC patients and reported that the exosomes span all chromosomes and contain DNA harboring mutated KRAS and p53. Mutated KRAS in exosomes is associated with a poor therapeutic response; therefore, it has great potential as a companion diagnostic marker for Erbitux [33]. However, KRAS mutation alone is not sufficient for PC diagnosis because of its low sensitivity and specificity, although it could be included in a tumor diagnostic test panel.

MicroRNAs

miRNAs are small non-coding RNAs that plays a role in post-transcriptional regulation by binding to target mRNAs to degrade or suppress their expression. Risheng Que et al found that exosomal miR-17-5p levels in serum were higher in PC patients than in non-PC patients and healthy participants. High levels of miR-17-5p correlated significantly with metastasis and advanced stage PC [32]. Madhavan et al reported that miR-1246, miR-4644, miR-3976, and miR-4306 were significantly upregulated in 83% of PC serum-exosomes, but rarely in control samples [35]. Moreover, Joshi et al used a localized surface plasmon resonance-based microRNA sensor to quantify miR-10b in highly pure exosomes isolated from patients with PC or chronic pancreatitis and from normal controls. The results showed that miR-10b levels were significantly higher in plasma-derived exosomes from PC patients than in those from patients with chronic pancreatitis or normal controls [37]. These findings suggested that miR-10b can be used as an early diagnostic biomarker for PC. At the same time, this unique technique can be used to design novel diagnostic strategies for pancreatic and other cancers based on direct quantitative measurement of microRNAs in plasma and exosomes, and it can be readily extended to other diseases with identifiable microRNA signatures.

MIF

As mentioned above, Costa-Silva et al [21] reported higher levels of plasma exosomal MIF in PC patients after diagnosis than in healthy individuals and patients with no evidence of disease. The study also suggests that exosomal MIF may be an attractive therapeutic target and a sensitive biomarker for PC. In the same year, Melo et al detected PGC1 on tumor-derived exosomes in serum; detection of PGC1-expressing exosomes in serum was 100% sensitive and specific for PC, including stage I [36]. Combining different systems for detecting detection exosomal PGC1 and MIF may provide an attractive noninvasive diagnostic tool to identify PC at the very early stages. Costa-Silva’s study further proposes MIF as a potential target for PC therapies; this may improve the dismal prognosis for PC.

Adrenomedullin

New-onset diabetes and concomitant weight loss occurring several months before the clinical presentation of PC appear to be paraneoplastic phenomena caused by tumor-secreted products. Gunisha Sagar et al reported that early-onset weight loss in PC patients (which preceded onset of PC-related symptoms) was due to PC-induced lipolysis mediated by exosomal adrenomedullin [40]. Their work provides insight into the early-onset paraneoplastic effects of PC and may help to identify targets and develop strategies for early detection of PC.

2.2 Exosomes as biomarkers for predicting treatment outcome

EGFR

Epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein [44]. Aberrant expression and mutation of EGFR promotes cell proliferation, tumorigenesis, metastasis, and resistance to radiochemotherapy [45]. Adamczyk et al reported the detailed mass spectrometry analysis of EGFR isoforms in secretomes from human PC cells. They identified 110 kDa soluble
N-terminal fragment of EGFR and two EGFR isoforms (a 170 kDa full-length receptor and a 65 kDa C-terminal remnant fragment isoform) in exosomes [46]. The US Food and Drug Administration has approved drugs such as Erlotinib [47], Cetuximab [48] and Gefitinib [49] as EGFR-targeting therapies. Soluble EGFR in exosomes may be a serum biomarker for predicting treatment outcome.

BRCA1/2

As tumor suppressors, breast cancer susceptibility genes BRAC1/2 play important roles in regulating transcription and DNA damage repair [50]. PC shows genomic instability and DNA defective damage repair mechanisms; these defects are partially due to mutation of BRCA1/2 [51]. San Lucas et al isolated exosomes shed into biofluids of three patients with pancreatobiliary cancers (two pancreatic and one ampullary) and performed comprehensive profiling of exoDNA and exoRNA by whole genome analysis. Multiple actionable mutations, including alterations in NOTCH1 and BRCA2, were identified in patient exoDNA samples [39]. To date, an inhibitor of poly (ADP-ribosyl) polymerase-1 (PARP1), olaparib, has shown some clinical benefit in patients harboring BRCA1/2 mutations [52,53]. Liquid biopsies containing shed exosomes have potential utility as a clinical tool for cancer diagnosis, therapeutic stratification, and treatment monitoring, thereby precluding the need for direct tumor sampling.

3 Exosome-based cancer therapeutics

Exosome-based therapies are an attractive approach to PC [54]. Because they are autologously generated within the host, exosomes can be engineered to carry drugs or target proteins without invoking an immunogenic response [55]. Thus, to successfully address and eradicate PC, it is imperative to develop therapeutic strategies that neutralize both cancer cells and exosomes simultaneously [56]. In the following section, we provide an overview of some of the different therapeutic applications of exosomes and discuss the future course of therapeutics with respect to PC [57].

Exosomes/curcumin

Curcumin, a derivative of turmeric root, has potent anticancer and anti-inflammatory effects both in vitro and in vivo. Osterman et al found that curcumin alters the role of PC exosomes from pro-survival to pro-death, resulting in reduced cell viability of PC cells; they suggested that exosomes may increase the efficacy of curcumin [56].

Exosomes/survivin-T34A

The inhibitor of apoptosis protein survivin is a key factor in maintaining resistance to apoptosis; it is blocked by its dominant-negative mutant (survivin-T34A), which activates caspases and triggers apoptosis. Aspe et al collected exosomes from a survivin-T34A melanoma cell line and plated them onto a PC cell line. They found that exosomes containing survivin-T34A induced a significant increase in apoptotic cell death when compared to gemcitabine. This exosome/survivin-T34A study describes a novel method of delivering anticancer proteins within the cancer microenvironment, which may prove useful for targeting cancers of the pancreas [58].

Loading exosomal carriers with therapeutic cargo

Exosomes released from drug-treated HepG2 cells show strong anti-proliferative effects on the human pancreatic cell line CFPAC-1 and induce immunogenicity and heat shock protein (HSP)-specific NK cell responses [59]. Moreover, Pascucci et al. observed that paclitaxel-treated mesenchymal stromal cells have strong anti-tumorigenic effects due to their capacity to take up the drug and release it later in exosomes. Paclitaxel-treated extracellular vesicles inhibited human PC cell proliferation in a dose-dependent manner, and inhibited tumor growth in vivo by 50% [60].

Pancreatic tumor nanoparticles (mimicked exosomes)
Of the many exciting developments in drug delivery technology, nanotechnology holds great promise as a targeted and controlled-release drug delivery platform. Ristorcelli et al developed pancreatic tumor nanoparticles that mimic exosomes (these particles were characterized by proteomic analyses and found to be rich in lipid rafts) and inhibit tumor cell proliferation [61]. These nano-exosomes increased proapoptotic factors, suppressed anti-apoptotic Bcl-2-induced PTEN and glycogen synthase kinase (GSK)-β activation, and decreased pyruvate dehydrogenase activity while concurrently suppressing β-catenin. The same group also showed that nano-formulations of exosomes induce PC cell death by inhibiting the Notch pathway [62,63].

4. Conclusions

PC has the highest mortality rate of all solid organ cancers. Even regular chemotherapy does not improve the survival rate significantly; therefore, new treatments/biomarkers are urgently needed. Exosomes contain a variety of molecules and may have important biological functions that facilitate PC spread. Here, we discussed recent research into PC-derived exosomes and listed potential biomarkers. Even though most studies are still at the laboratory stage, we believe that advances in molecular biological techniques and studies involving of larger cohorts will result in the discovery of novel and effective therapeutic approaches that will improve both the quality of life and prognosis of patients with PC.

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Abbreviations

PC pancreatic cancer
CD44v6 CD44 variant isoform v6
CICs Cancer-initiating cells
CAFs Cancer-associated fibroblasts
GIPC GAIP-interacting protein C terminus
GPC1 glypican-1
EGFR Epidermal growth factor receptor
PARP1 Poly (ADP-ribosyl) polymerase-1
GSK glycogen synthase kinase

References


