Cancer Exosomes for Early Pancreatic Cancer Diagnosis and Role in Metastasis

Murray Korc and Samantha Deitz McElyea

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a treatment-recalcitrant and highly metastatic cancer. Recent studies have demonstrated that PDAC is associated with an increased release of small vesicles called exosomes that are ~40 to 130 nanometers in diameter. These exosomes may derive from pancreatic cancer cells, cancer-associated fibroblasts, and infiltrating immune and inflammatory cells. They carry a cargo rich in proteins, lipids, DNA, and microRNAs. Exosomes can modulate the tumor microenvironment, promote pancreatic cancer cell proliferation, invasion, and metastasis, and prime the pre-metastatic niche to facilitate formation of distant metastatic lesions. Components of the exosomal cargo may also serve as diagnostic biomarkers and guide the design of precision medicine strategies. Finally, exosomes have been proposed to act as biological nanoparticles that can be loaded with drugs for therapeutic use.

Keywords

Early diagnosis • MicroRNAs • Exosomes • Metastasis

Contents

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1 Exosomes

1.1 Unique Origin of Exosomes

Exosomes are ~40 to 130 nanometer (nM) particles of endosomal origin that derive from multivesicular bodies (MVBs) from which they are released following MVB fusion with the cell membrane [1, 2], as shown in Fig. 1. Consequently, they express endosomal proteins, including tumor susceptibility gene 101 (Tsg101) and Alix [1, 2], as well as many other proteins. By contrast to exosomes, microvesicles are shed directly from the cell membrane and are ~50 to 1000 nM in diameter, whereas apoptotic bodies are generated by the release of membrane blebs from cells undergoing apoptosis, and their diameter ranges in size form ~800 to 5000 nm [3–5].

It has been known for several decades that cells have the capacity to internalize fluids, large molecules, plasma membrane fragments, extracellular ligands, and cell-surface receptors through a process called endocytosis [6]. The internalized material ends up in multiple endocytic vesicles that undergo fusion to form the early endosome compartment. Some of this internalized material, including signaling receptors, can recycle from the early endosome to the cell surface, whereas other components are transported to the late endosomal pathway and subsequently to lysosomes where they undergo degradation [6].

Receptor-mediated endocytosis may occur through clathrin-coated pits on the cell membrane, and through clathrin-independent mechanisms, and is under complex regulatory control [7, 8]. Importantly, ligand-receptor dissociation occurs in the late endosome, whereas these complexes often remain intact in the early endosome and can continue to signal [9]. Since exosomes derive from MVBs that had formed from endosomes, exosomes also have the capacity to carry and deliver the internalized receptors to target cells where, in theory, they may participate in signaling events [9–11]. Conversely, growth factor receptor signaling can act to promote late endosome formation [12], suggesting that this compartmentalization mechanism serves to fine tune receptor-mediated signaling output.

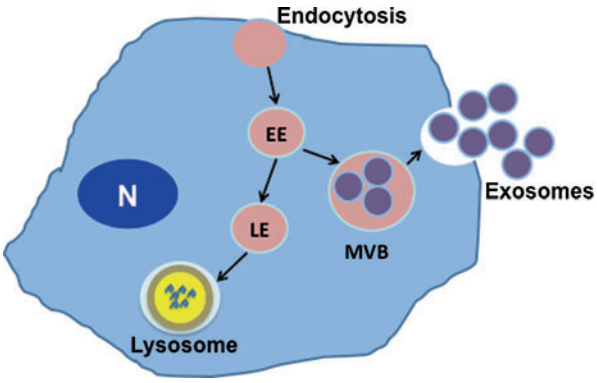

Fig. 1 Schematic representation of exosome formation. A cancer cell is shown exhibiting endocytosis followed by formation of an early endosome (EE) that can progress to become a late endosome (LE) or end up in a multivesicular body (MVB). Fusion of the MVB with the cell membrane leads to the release of exosomes. Most late endosomes end up in lysosomes where they undergo degradation. N: nucleus

1.2 Exosome Isolation Methods

Exosomes can be isolated from all bodily fluids, including blood, urine, and saliva. When seeking to study exosomes in the circulation, serum or plasma can be prepared by using red top or lavender top collection tubes, respectively. Lavender top collection tubes are coated with EDTA, which does not interfere with microRNA (miRNA) analysis.

Specimens should be promptly placed on ice or in a refrigerator (4 °C), taken to the lab within less than 60 min, and rapidly centrifuged (1000 x *g* for 10 min) at 4 °C. Supernatants can then be collected and stored at −80 °C until it is time to prepare the exosomes [13, 14], or subjected to a second optional centrifugation (10,000 x *g* for 10 min) at 4 °C to ensure the removal of any residual coarse debris prior to storage at −80 °C. To prepare exosomes, samples should be thawed on ice, centrifuged at 10,000 x *g* for 30 min (4 °C), and filtered through a 0.22 μm filter to remove remaining debris. Appropriate aliquots (for example, 250 μl/sample) can then be centrifuged at 110,000 x *g* for 2 h (4 °C). The resulting pellets should be washed with phosphate-buffered saline (PBS) to remove debris, and then resuspended in PBS prior to undergoing a second 110,000 x *g* centrifugation for 2 h (4 °C).

There are a variety of alternate methods for isolating exosomes [15–17]. A few examples include sucrose gradient fractionation, size-exclusion chromatography, affinity chromatography, affinity immunoprecipitation, polymer-based precipitation as described in System Bioscience's protocol (https://www.systembio.com/downloads/Manual_ExoTC_WEB.pdf), and immunoaffinity capture [14, 15]. An example of the latter method is based on the observation that exosome extraction from solutions can be accomplished through their binding to bead-immobilized Tim4 via their surface phosphatidylserine (PS) [16]. Given that Tim4 binding to PS is Ca^{2+}-dependent, the captured exosomes can be released from the magnetic beads by Ca^{2+} chelation [16].

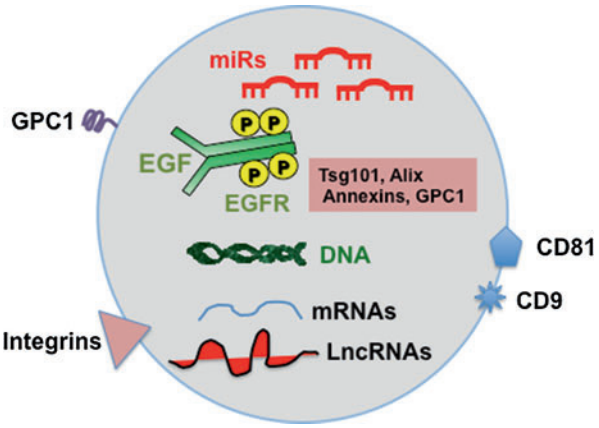

Fig. 2 Schematic representation of an exosome. The cholesterol-rich membrane of an exosome is shown decorated with the glycophosphatidylinositol-anchored glypican-1 (GPC1), the tetraspanins CD9 and CD81, and integrins. The cargo within the lipid bilayer of the exosome includes microRNAs (miRs), DNA, mRNAs, and long noncoding RNAs (LncRNAs). Cargo proteins include the EGF receptor (EGFR), TSG101, Alix, and GPC1, among many others

When studying exosomes released by cells during cell culture, it is important to remember that serum, including fetal bovine serum (FBS), contains exosomes and that these exosomes should be removed prior to use in cell culture studies. Alternatively, exosome-free FBS is commercially available.

1.3 Exosome Characteristics

Exosomes are vesicles consisting of a single membrane phospholipid bilayer with both surface and embedded proteins (Fig. 2). In addition to expressing endosomal proteins, exosomes are enriched for proteins deriving from cell membrane domains that tend to undergo internalization to form endosomes, such as tetraspanins, lipid-protein raft components, and adhesion molecules [17, 18]. Tetraspanins have four transmembrane domains and may be highly abundant in certain exosomes, depending on their cell of origin [3]. Exosomes are also rich in flotillins, which are involved in endocytosis [15], and annexins, which are phospholipid-binding proteins that are regulated by calcium and that have been implicated in the modulation of numerous cell processes including exocytosis [19].

Exosomes also tend to be enriched for proteins that are anchored to the cell membrane via a glycosylphosphatidylinositol (GPI) motif [20]. For example, the heparin sulfate proteoglycan (HSPG) glypican-1 (GPC1) is overexpressed in PDAC and is a GPI anchored protein [21] that contributes to PDAC angiogenesis and pancreatic cancer cell proliferation [22–24]. HSPGs are ubiquitous cell surface molecules consisting of core proteins covalently linked to glycosaminoglycans (GAGs) polysaccharide chains that are characterized by disaccharide repeats such

as L-iduronic or D-glucuronic acid and either N-acetylgalactosamine or N-acetylglucosamine [25, 26]. GAGs may consist of heparin and heparan sulfate (HS), chondroitin or dermatan sulfates, as well as hyaluronan or keratan [26, 27]. Importantly HSPGs act as receptors that internalize exosomes [28] and HSPGs are also taken up by exosomes [28]. Therefore, it is not surprising that GPC1 was recently shown to be present in exosomes from patients with PDAC, normal control subjects, and patients with chronic pancreatitis [29]. The manifold components of the cargo of exosomes can be found in the ExoCarta database (http://www.exocarta.org).

2 Pancreatic Cancer and Exosomes and Diagnostic Potential

2.1 Size Distribution of Pancreatic Cancer Exosomes

Several techniques are generally used to assess the quality and size of the exosome preparation. Visualization by electron microscopy provides strong confirmatory evidence for the purity of the exosome preparation. However, alterations caused by sample fixation or dessication, or by exosome adherence to the template surface can alter the shape and apparent diameter of the exosomes [30]. Moreover, electron microscopy is both expensive and time consuming. Alternate techniques for assessing exosome size and number are generally used by many laboratories [31]. For example, the size distribution and concentration of exosomes in a biological fluid can be determined with readily available instruments. Thus, nanoparticle tracking analysis (NTA) allows for the measurement of particle size by determining the angular variation in intensity of scattered light following laser illumination [32]. NTA is readily performed in a reproducible manner by instruments manufactured by Nanosight [33]. By contrast, the qNano system uses a nanopore and tunable resistive pulse sensing to quantitate particle size and concentration [33].

Using the qNano system, a recent study evaluated the size of exosomes from normal controls, patients with PDAC, and patients with chronic pancreatitis [28]. The diameter of the majority of normal control exosomes ranged from 60 to 100 nm. By contrast, the diameter of CP and PDAC exosomes ranged from 70 to 120 nm [28]. However, only the PDAC-derived exosomes had numerous exosomes that ranged in size from 85 to 115 nm (Fig. 3a). Importantly, within 24 h following PDAC resection the diameter of the PDAC-derived exosomes in the circulation reverted to the diameter in control samples (Fig. 3b). These observations suggest that high levels of 85–115 nm range exosomes point to the presence of an underlying PDAC and that these exosomes contain an altered, cancer-associated cargo.

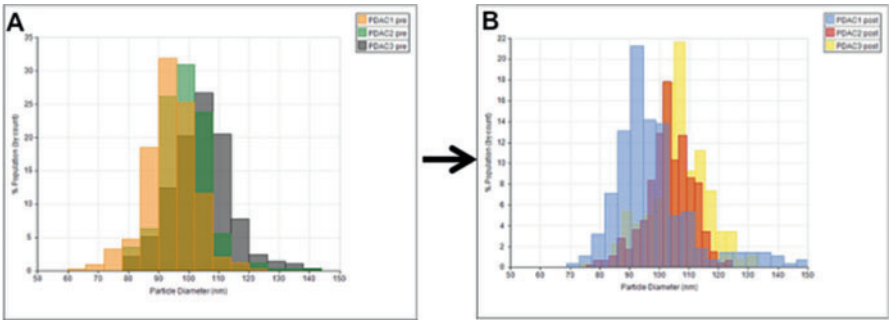

Fig. 3 Exosome distribution pre- and post-resection. Exosome diameter was determined using the qNano system. (**a**) Size distribution of exosomes prior to pancreatic cancer resection, when exosomes were mostly in the 85–115 nm range. (**b**) Size distribution of exosomes 24 h following PDAC resection was similar to that observed in control samples and in chronic pancreatitis samples. Data are from reference 28

2.2 Content of Pancreatic Cancer Exosomes and Diagnostic Utility

Studies with exosomes in pancreatic cancer patients have mostly relied on exosome isolation from serum or plasma, but PDAC-derived exosomes can also be found in saliva [34] and potentially other bodily fluids such as ascites, bile juice, and pancreatic juice. In the case of urinary exosomes, it has been demonstrated that following immunocapture on magnetic beads, it is possible to rapidly trypsinize the outer exosome proteins on the beads and identify them by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis [35].

Irrespective of their source, exosomes are stable in the circulation. However, in addition to their elimination into the urine, exosomes are also removed from the circulation due to their uptake by different cell types in various organs, as demonstrated in studies with miRNA-155-loaded exosomes that were injected into miRNA-155 knockout mice [36] and with fusion protein engineered for intra-exosomal expression and that had been labeled with radioactive iodine [37]. It is therefore likely that a steady state exists between exosome release into the circulation and exosome uptake in peripheral tissues and clearance by other means, which dictates the number and source of exosomes present in the blood. Nonetheless, the cargo within the exosome is protected from degradation, and this characteristic feature of exosomes enhances their diagnostic utility in disease states.

In addition to a complex intra-exosomal cargo that consists of proteins, miRNAs, long non-coding RNAs, mRNAs, transfer RNAs, lipids, and double-stranded DNAs that have been shown to derive from all human chromosomes [5, 19, 35], PDAC-derived exosomes can carry mutated *KRAS* and *TP53* DNA, reflecting the mutation profile in the specific PDACs from which the exosomes were released [38]. In theory, therefore, it should be possible to establish signatures based on a mix of these biomarkers to confirm PDAC diagnosis and to monitor response to therapy,

and ultimately to diagnose the disease at an early and resectable stage. For example, the combination of the levels of proteins, such as CD104, EpCAM, and Tspan8, together with the levels of miRNAs such as miRNA-1246, miRNA-4644, miRNA-3976, and miRNA-4306 has been reported to constitute a sensitive and specific signature for PDAC [39]. However, sensitivity and specificity issues remain to be addressed for many PDAC biomarker studies, even in relation to DNA mutation analysis [40].

Importantly, to date, early PDAC detection has remained an elusive goal [41]. Yet, it is widely accepted that noninvasive, informative biomarkers for early PDAC diagnosis are a major unmet need that have the potential to aid considerably in prolonging survival in this patient population. Although a well-executed recent study using an anti-GPC1 antibody reported that exosomal GPC1 may be diagnostic for early PDAC with high sensitivity and specificity [42], another study, using LC-MS/MS, revealed the presence of GPC1 in exosomes from normal controls and from patients with chronic pancreatitis, with overlap in exosomal GPC1 levels between the three groups [28]. The differences between the two studies could be due to the different methods used to detect this HSPG. Thus, one study used an anti-GPC1 antibody [42] and such an antibody could be directed against an aberrant glycan epitope that in theory could be cancer-specific. By contrast, the LC-MS/MS method used in the second study [28] measured the core protein and is not influenced by the glycanantion status of GPC1.

Exosomes from patients with PDAC carry high levels of miRNA-10b, miRNA-21, miRNA-30c, whereas the exosomal levels of all three miRNAs are low in normal control subjects or in patients with chronic pancreatitis [28]. Moreover, the elevated levels of all three miRNAs are greatly decreased at 24 h following PDAC resection, underscoring their PDAC origin [28]. It remains to be determined, however, whether a combined exosome and plasma signature could yield sensitive and specific biomarkers for early PDAC diagnosis and for monitoring PDAC recurrence following resection.

Given the clinical implications and potential benefits of early PDAC diagnosis, there has been a great deal of effort to devise advanced technologies to facilitate exosome analysis. For example, to enrich for PCC-derived microvesicles circulating in the plasma, Liang et al. designed a sensor chip that was coated with an antibody that targets the tetraspanin CD81, leading to the highly efficient capture and immobilization of microvesicles directly from plasma [43]. PCCs are believed to preferentially release exosomes (50–100 nm) over other microvesicles [44], so presumably, when using plasma from PDAC patients, this chip would mostly retain exosomes.

The captured vesicles were hybridized with gold nanospheres and nanorods that have been conjugated with antibodies against membrane-bound erythropoietin-producing hepatocellular receptor tyrosine kinase class A2 (EphA2), and anti-CD9, respectively [43]. The use of gold nanoparticles provided two key advantages. First, the inertness of gold nanoparticles prevented spurious interactions. Second, gold nanoparticles are known to exhibit robust localized surface plasmon resonance, and the use of two different types of antibody-conjugated nanoparticles (nanospheres and nanorods) enhanced both the sensitivity and specificity of the assay due an increased

intensity of the plasmon resonance and a readily detectable wavelength shift [43]. Using this nanoplasmon enhanced scattering (nPES) assay, Liang et al. showed that they can differentiate the high EphA2 vesicle signal from PDAC patients by comparison with corresponding signal from normal controls and chronic pancreatitis patients.

It will be important to confirm that specificity and sensitivity of the nPES assay will remain high as more samples are examined. It is also not clear whether this approach could be used to diagnose microscopic PDAC or early Stage IA disease. Finally, it should be noted that PDAC is rich in cancer-associated fibroblasts (CAFs), and these CAFs also release exosomes. It would therefore be interesting to determine whether the anti-CD81-coated sensor chip is also able to capture CAF-derived exosomes.

3 Pathological Actions of Exosomes in Pancreatic Cancer

3.1 Exosome Actions in the Tumor Microenvironment (TME)

Lung cancer cells release exosomes that carry miRNA-21 and miRNA-29a, and these exosomes bind Toll-like receptor 7 (TLR7) and TLR8 in immune cells, thereby eliciting an inflammatory response that enhances cancer cell proliferation and metastasis, revealing a novel role for exosomes in the TME [45]. Inasmuch as miRNA-21 is abundant in PDAC-derived exosomes [28], it is possible that a similar phenomenon may occur in the TME in PDAC. Additional potential actions in the TME include the modulation of oxidative phosphorylation or glycolysis by both cancer cell-derived and CAF-derived exosomes due, in part, to the inhibition mitochondrial oxidative phosphorylation and providing an energy supply to the cancer cells through the exosomal cargo of amino acids and lipids [46, 47].

It is possible that exosomes within the TME may also transfer to noncancerous cells mutant Kras protein and tyrosine kinase receptors such as the epidermal growth factor (EGF) receptor (EGFR) and ligands that bind to EGFR, such as amphiregulin, thereby creating a field effect that nurtures tumor growth [48–50]. In addition, through their stimulatory effects on the conversion of pancreatic stellate cells (PSCs) into CAFs, exosomes can act to enhance PDAC desmosplasia, and through their ability to induce EMT and inhibit cancer-directed immune pathways, exosomes can promote PDAC metastasis [51, 52]. Exosomes also contain proteases and can therefore degrade components of the extracellular matrix such as collagens, fibronectin, and laminins [53]. In turn, ECM degradation liberates growth factors and matrix metalloproteases that combine to exert mitogenic, motogenic, and invasion promoting effects on the PCCs while also activating pro-survival pathways and apoptosis resistance in the PCCs.

3.2 PDAC-Associated Exosomopathies

PDAC may be associated with systemic prodromal manifestations that appear prior to the cancer diagnosis. Such prodromal syndromes include pancreatogenic diabetes, which is also known as type 3c diabetes mellitus or T3cDM diabetes [43, 54], unexplained weight loss, which could be viewed as a pre-cachexia state, and thromboembolic events that have also been described as Trousseau syndrome.

The mechanisms underlying T3cDM have not been completely delineated but include resistance to insulin actions. In addition, a recent study [43] reported that one mechanism for T3cDM is the release by the PCCs in PDAC of exosomes that carry adrenomedullin (AM). Following release into the systemic circulation these exosomes return to the pancreas, enter the islets, and interact with the β-cells where AM delivery induces an unfolded protein response that interferes with β-cell function and may even lead to β-cell death [43]. Consequently, these patients cannot mount a robust insulin response to their insulin-resistant state, and therefore exhibit rising blood glucose levels. Thus, T3cDM can be viewed as an exosomopathy [55].

In spite of a great deal of progress in understanding the multiple pathways that contribute to cancer cachexia [56], the potential role of exosomes in PDAC-associated cachexia is yet to be fully explored. Nonetheless, it is now recognized that microvesicles from PC1, Panc-2, and MIA PaCa 2 PCCs carrying miRNA-21 can signal through TLR7 in Pax7-positive murine myoblasts to activate c-Jun N-terminal kinase and promote muscle cell apoptosis [57]. By contrast, similar myoblasts prepared from TLR7$^{-/-}$ mice were resistant to apoptosis when exposed to either conditioned medium samples from Lewis lung carcinoma cells that induce cachexia in mice, or to serum samples from 5 of 7 pancreatic cancer patients who were diagnosed as having cancer cachexia [57]. Of note, Pax7 is a transcription factor expressed in the nuclei of muscle stem cells that controls their self-renewal. Previously, it was shown that its persistent expression in muscle stem cells during cachexia prevents them from differentiating into adult muscle cells and/or from fusing to damaged myofibers and thus impedes myofiber repair and promotes muscle atrophy [58]. It remains to be determined whether such a mechanism is active in relatively early stages of PDAC, whether TLR8 in humans mediates the same pathways as TLR7 to induce muscle stem cell apoptosis, and what ultimately dictates the fate of these Pax7-positive satellite cells between failure to differentiate vs. apoptosis.

With respect to the third type of exosomopathy, while the underlying etiologies in venous thromboembolic events in PDAC are not well understood, they have been correlated with elevated plasma Tissue Factor (TF) levels [59]. This correlation has also been observed in a mouse xenograft model of PDAC [60]. TF is carried by microparticles [61], and therefore may also be carried by exosomes. However, in the case of melanoma cells, it was shown that most of the TF is found in microvesicles and apoptotic bodies [62]. Nonetheless, monocyte-derived exosomes have been shown to exert pro-thrombotic actions [63]. Given that thromboembolic events are an important cause of death in cancer patients receiving chemotherapy as outpatients [64], this aspect of PDAC pathobiology needs further exploration.

3.3 Exosomes and the Metastatic Niche

The metastatic process consists of a complex sequence of events that includes different types of cancer cell migration, EMT, invasion, immune alterations within the TME, and, systemically, extracellular matrix degradation, breaching of barriers in a manner that enables the cancer cells to enter into blood vessels and lymphatics, survival of these cells in the circulation, and successful colonization of distant organs within a receptive microenvironment called the metastatic niche [65–67]. Recent studies have highlighted the important role of miRNAs in cancer progression and metastasis due to their ability to regulate cell proliferation, migration, invasion, and metastasis [68–73].

Exosomes can promote cancer metastasis by carrying deleterious miRNA and proteases to distal sites where they prime the normal microenvironment to be receptive to circulating cancer cells [53, 74]. In addition, exosomes can target immune pathways in a manner that promotes the metastatic process. For example, exosomes have been reported to exert effects on the distant "soil" by priming the pre-metastatic niche to be receptive to metastatic cells. Exosomes were also suggested to increase the number of myeloid derived suppressor cells (MDSCs) in the TME which leads to the release of inflammatory cytokines that in turn prime the pre-metastatic niche [75].

Costa-Silva et al. performed a crucial study that definitively demonstrated an important role for PDAC-derived exosomes in priming the pre-metastatic niche in the liver [76]. They showed that purified exosomes from different murine models of PDAC (5 μg exosomes per injection) every other day for 3 weeks prime the hepatic pre-metastatic niche to be receptive to intrasplenic injections of PCCs, yielding macro-metastatic lesions 3 weeks later. Mechanistically, they demonstrated that the exosomes are taken up by the Kupffer cells and induce transforming growth factor β (TGF-β) expression that in turn upregulates fibronectin production by hepatic stellate cells [76]. The abundant fibronectin leads to the recruitment and retention of bone marrow–derived macrophages. Importantly, exosomal macrophage migration inhibitory factor (MIF) was required for TGF-β upregulation, and in the absence of MIF or following macrophage ablation, the exosomes no longer exerted a pro-metastatic effect. Clinically, exosomal MIF levels were higher in stage I PDAC patients that eventually developed hepatic metastases by comparison to stage I patients who did not develop such lesions. As pointed out in an accompanying commentary [77], other exosomal components may also be important for the metastatic process both in terms of metastatic sites and in terms of PCC proliferation, which may also include unrecognized intrinsic characteristics of the PCCs. These findings are also in agreement with a long-standing observation that high levels of TGF-β isoforms in PDAC patients who had resectable disease and who did not receive any post-operative therapies were associated with earlier disease recurrence and shorter overall survival by comparison with patients whose PDAC expressed low levels of these isoforms [78]. It may therefore be timely to reconsider the reluctance to target TGF-β in clinical trials in patients with PDAC.

4 Therapeutic Implications

4.1 Exosomes for Drug Delivery

Initial efforts at using exosomes as drug delivery "nanoparticles" began in the past several years. For example, Alvarez-Erviti et al. reported that it is possible to deliver short interfering RNAs (siRNAs) to the mouse brain by systemic injection of targeted exosomes, based on the fact that exosomes cross the blood-brain barrier [79]. The exosomes were self-derived from dendritic cells, thereby avoiding any possible immune reactions against foreign exosomes, and were targeted to the central nervous system by engineering a fusion of lamp2b to neuron-specific RVG peptide [79].

In a subsequent study, it was shown that exosomes can be targeted to breast cancer cells expressing high EGFR levels by engineering the cells to express the GE11 peptide that is known to bind to EGFR, and making sure that expression is directed to the cell membrane by using a vector expressing the transmembrane domain of the platelet-derived growth factor receptor [80]. The authors then showed that the intravenous injection of exosomes from the engineered cells can deliver miRNA let-7a to breast cancer xenografts [80]. A variety of other strategies have been proposed to improve exosomes as drug delivery vehicles [81], and a more recent study demonstrated that it is possible to engineer exosomes to express a single domain antibody (nanobody) against EGFR using a GPI-based anchoring strategy, thereby greatly enhancing exosome delivery to EGFR overexpressing cells [82].

A novel therapeutic approach for PDAC was recently proposed based on the fact that oncogenic *KRAS* is the major truncal mutation in this malignancy and that the mutant Kras protein has been resistant to targeting. Using exosomes derived from normal fibroblast-like mesenchymal cells that were engineered to express either the siRNA or the hairpin RNA (shRNA) that specifically downregulate $Kras^{G12D}$, the most common type of mutated *KRAS* in PDAC, Kamerkar et al. demonstrated dramatic efficacy in several mouse models of PDAC [83]. It seems likely that this strategy will also be successful with other forms of mutant Kras, but this remains to be demonstrated.

5 Conclusion

It is likely that following up on the advances of recent years, it will be possible to develop novel strategies for early PDAC diagnosis that are based on the analysis of exosome cargo and that this approach will also yield novel prognostic markers. Moreover, advances in DNA mutational analysis of exosomal DNA could guide novel precision medicine approaches and the rapid monitoring of therapeutic responses. Exosomes will also be increasingly useful as drug delivery vehicles and as positive modulators for activating cancer-directed immune mechanisms. The combination of these new tools will likely dramatically improve the survival of patients with PDAC.

6 Key Research Points

- Exosomes are important intracellular regulatory vesicles within their cells of origin.
- Exosomes are released into their microenvironment and biological fluids, where they regulate numerous cellular processes.
- In PDAC, exosomes may exert effects on the tumor microenvironment to enhance PDAC growth and metastasis and to prime the pre-metastatic niche.

7 Future Scientific Directions

- There is a need for improved and highly reproducible assays of exosome content.
- There is a need for strategies to promote beneficial effects of exosomes and to block their deleterious effects.
- There is a need to understand how to modify endogenous exosomes for therapeutic purposes.
- There is a need to gain a better understanding of the therapeutic applications of exogenous exosomes.

8 Clinical Implications

- Exosome cargo can be analyzed to establish diagnostic and/or prognostic signatures in PDAC.
- Exosomes can be used as therapeutic vehicles.

Specific medical conditions associated with PDAC such T3cDM, cachexia, and venous thromboembolic event may be aggravate by exosomes and targeting these exosomes could lead to improved survival and improved quality of life in patients with PDAC.

9 Cross-References

► Clinical Applications of Genomics and Proteomics in Pancreatic Cancer
► Development of Novel Diagnostic Pancreatic Tumor Biomarkers 2nd ed
► Development of Novel Therapeutic Response Biomarkers
► Diagnostic Biomarkers
► Emerging Therapeutic Targets in Pancreatic Adenocarcinoma
► Paraneoplastic Syndromes in Pancreatic Cancer
► Precision Medicine Based on Next Generation Sequencing and Master Controllers

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