

Special Issue

The Role of Exosomal Cargo in the Regulation of the Biological Complexity of Pancreatic Cancer

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Abstract

Pancreatic cancer is among the deadliest of all human cancers and remains incurable. There is an urgent need for the identification of specific diagnostic and therapeutic biomarkers with hopes that such markers could be targeted in the development of novel therapeutics for better treatment of pancreatic cancer. The concept that extracellular transport can be mediated by vesicular structure especially exosome was brought to the scientific community in the 1980s; however, it was only in the last 10 years that the field of exosome biology has gained momentum. The surge in research interest was supported by the realization that these vesicular structure can participate in several important cellular processes, and in cancer, these small vesicles are emerging to be recognized as powerful modulators of most of the well-known cancer hallmarks. Exosomes serve as important vehicle for delivering cargo containing proteins, lipids, and nucleic acids. They have been shown to mediate intercellular communication between different cell types in the body, and thus affecting the functioning of normal homeostasis as well as the functioning of different pathological conditions. This brief editorial touches upon some of the complex but expanding role of exosomal cargo in mediating the biology of pancreatic cancer. Some perspective is provided as to how the field of pancreatic cancer in the context of exosome is taking shape with the hope to bring forward successful clinical applications of exosome in guiding advancement for the treatment of pancreatic cancer.

Keywords: Pancreatic cancer; Pancreatic ductal adenocarcinoma; Exosomes; Microvesicles; Cancer; Drug resistance; Microenvironment; Carcinogenesis; Cancer hallmarks; MicroRNAs; Therapeutics; Clinical trials

Introduction

Pancreatic cancer remains a deadly disease that kills approximately 40,000 Americans each year making it the fourth leading cause of cancer related deaths in the United States [1]. Pancreatic cancer patients lack early detection markers, and actionable therapeutic biomarkers. There is no effective therapy available to tame this disease, and the diagnosis of pancreatic cancer is often considered a death sentence. Therefore, there is an urgent need for the identification of early diagnostic biomarkers and novel therapies in order to positively impact the dismal statistics associated with pancreatic cancer.

The last ten years have seen an unprecedented spurt of research work in the field of extracellular vesicles [2]. Majority of this work has focused essentially on certain specific type of extracellular vesicles originating from the endosomes and termed as exosomes [3]. These tiny vesicles have a stable membranous covering in which is embedded a very complex payload (cargo) composed of immune molecules, sugars, steroids and nucleic acids [2,4]. The exosomes are released from a wide variety of cell types that includes normal and cancer cells [5]. The content of an exosome is considered as a reflection of its originating cell as it carries very specific traits from the parental cell [6]. In essence, the secreted exosomes are representative of the metabolic status of their originating cells. Exosomes has been well documented to play important role in cancer development, metastasis and drug resistance [7]. Given that these vesicles are fingerprints of their originators' biological setup certainly holds significance in better understanding of the complexities of cancer. A number of important characteristics has been discovered which makes exosomes as ideal carrier of messages for different purposes such as diagnostics [8], understanding their role in inherent drug resistance [9], its role in tumor aggressiveness [10], in sustaining the cancer associated microenvironment [11] and also in the exploration for developing therapeutics against cancer [12]. Firstly they are actively secreted by live cells including tumor cells, and secondly their content reflects the originating tumor state and its microenvironment. Thirdly, the exosomes can be collected with simplistic purification methods from a wide range of bio-fluids using techniques that are less invasive. These exosomes are also found in high amount in ubiquitous proteins present in biological fluids, and remain very stable for extended period of time. This gives enormous flexibility to study the exosome over extended periods of time in all aspect of cancer development and progression.

The structure and content of exosomes have been parsed in great detail over the last decade. Thanks to advanced imaging tools, researchers have been able to glean through these intriguing vesicular structures at previously unfathomable depths. Their size has been conclusively shown to range between 30–150 nm although exceptions do occur. In regards to the contents, exosomal membrane has been shown to harbor, lipids, immunoglobulins, MHC complex, polysaccharides among other important biological macromolecules [13]. However, it is their payload, i.e., cargo that has been touted to have immense potential to influence the cellular microenvironment. This payload has been studied to impact and cause multiple effects that can result in initiation, progression and spread of cancer [7]. Exosomes have been shown to guide the export of major cancer regulatory proteins and transcription factors to the outer-cellular

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milieu. These proteins are either tumor promoters or tumor suppressors. Such exosomal secretion of proteins is expected to impact distant cell signaling or promote a niche that sustains tumor microenvironment leading to the spread of cancer especially by corrupting the entire tumor niche. The list of well recognized cargo's that exosomes carries and deliver to other cells includes p53, APC, Hsps, PTEN, Ras among others [14]. These proteins are well known players in the regulation of different cancer hallmarks such: sustained proliferation, insensitivity to anti-growth signals, replicative immortality, sustained angiogenesis, tissue invasion and metastasis. The exosomal content have also been shown to influence cancer associated deregulation of metabolism, promotion of immune evasion, cause chromosomal instability, and feed into the drivers of tumor promoting inflammation, The EXOCARTA database (a database that lists studies on exosomal cargoes) presently lists around 10,000 proteins as exosomal cargo's published to date has been reported (Table 1) [15].

Number of studies listed on PubMed when searched using keywords Exosomes	2991
Protein entries	41860
Proteins	9769
mRNA entries	4946
mRNA	3408
microRNAs	2838
Lipids	1116

Table 1: Updated complete list of EXOCARTA.

The discovery that along with proteins, the exosomes also carries and deliver small non-coding RNAs or microRNAs (miRNAs) which added a new dimension to this already complex field of vesicular research [16]. This is simply because a single miRNA can influence multiple genes and their distribution through exosomes can principally influence a cascade of events at sites that are far from their origin [17]. The number of secretory miRNAs captured in vesicular structures has skyrocketed in recent years. Research shows that vesicle miRNA loading is not random event; rather it is guided by specific set of proteins in a very selective and coordinated manner [18]. This results in only specific miRNAs being taken up by the exosomes and its delivery in the biologically desired setting. This selectivity has driven the field of cancer diagnostics, and the researchers are intensely searching for secretory miRNA signatures that can be applied to early detection or even for differentiating between benign and aggressive cancers [19]. In parallel, approaches are being developed to use these vesicular miRNAs as potent therapeutics to specifically silencing genes of interest at target site [20]. Given that the exosomes do not induce an immunogenic response, they can be used as ideal carriers of biological cargo especially for miRNA delivery without any unwanted toxicity that is usually associated with any other type of chemically generated delivery system.

Studies from intestinal epithelium has demonstrated that certain foods especially fruits and vegetables secrete exosome like structures as well [21-23]. This observation has driven research in the area of dietary exosomes, and thus there is considerable focus in the identification of secretary vesicles from different food sources which may ultimately regulate normal biological homeostasis in humans. This is supported by the findings that exosomes can mediate interspecies communication between plant and mouse gut host cells [24]. In fact, grape skin extracted exosomes are being exploited to induce cancer cell selective death in vitro [25]. Similarly, another natural dietary agent curcumin has been shown to induce the exosomes/microvesicles secretion that attenuates lysosomal cholesterol traffic impairment [26] and also attenuate cancer cell growth through a miRNA (miR-2) mediated mechanism of action [27]. These studies forms the principles for delivery of drugs or miRNAs using vesicles that are generated in an autologous manner thereby avoiding the toxicity related issues that usually are associated with nano-based carriers.

Clinical studies that either use exosomes for diagnostics or therapeutic purposes have steadily increased in the last few years. At present 19 clinical trials are listed on clincialtrial.gov in relation to exosomes, indicating that the concepts in vesicular research have some translational potential for various diseases including cancers. These clinical studies include the use of exosomes as biomarker for cancer NCT02439008, diagnostics (NCT02464930; NCT01344109; NCT02310451), therapeutic response to treatments (NCT02071719; NCT02507583) and in drug delivery (NCT01294072). Most of these studies are under active recruitment stage. It is anticipated that in the coming decade data from these and newer studies will be available which will give a clearer picture of success of exosome based approaches in the early detection or treatment of cancer. In the next paragraph, discussion will remain focused on the role of exosomal cargo in pancreatic cancer.

Exosomes in Pancreatic Cancer

Pancreatic cancer has complex biology that is dependent on the interaction of myriad signaling mechanisms [28]. The unanswered question remains, how so many of these different signaling mechanisms drive one common goal; vis a vis the development and sustenance of pancreatic cancer has intrigued researchers for many decades. Both localized and distant signaling interactions have been shown to play critical roles in sustaining the pancreatic tumor niche or the microenvironment. Such crosstalk has been postulated to occur through mobile carriers and that is where the exosomal mediated delivery of its cargo becomes the essence of scientific curiosity.

Earlier studied have shown the role of tetraspanins (a major constituent of exosome structure) in promoting pancreatic cancer metastasis [29]. Building on this initial description, many different groups evaluated the roles of vesicles and exosomes in understanding the biology of pancreatic cancer. For example, Gesierich et al., demonstrated a systemic induction of angiogenesis by tetraspanins in

gastrointestinal cancers particularly pancreatic cancer [30]. Similarly, Ristorcelli et al. have established a clear link showing that exosome can mediate apoptosis in pancreatic cancer cells through targeting of Notch signaling [31,32]. Researchers have also identified doublestranded genomic DNA spanning all chromosomes with mutated K-Ras and p53 DNA in the serum exosomes of patients with pancreatic cancer. Adamczyk et al. later characterized the released EGFR from pancreatic cancer cells which provided support for the concept that the receptor signaling could be under the influence of exosomal cargo [33]. Adding on to these studies, Arscott et al. proposed the role of EGFR isoforms in exosomes as novel biomarker resource in pancreatic cancer [34]. Another study showed that tumor exosomes can influence leukocyte activation thereby initiating an ambivalent signaling crosstalk in pancreatic cancer microenvironment [35]. In yet another study, Tspan8, CD44v6 and alpha6beta4 (all components of exosomes) were shown to be biomarkers of migrating pancreatic cancer-initiating cells [36]. The tetraspanins CD151 and Tspan8 have also been shown to guide the crosstalk between pancreatic cancer initiating cells and their surroundings [37]. More recently, Costa-Silva et al. showed that pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver [38-41]. In yet another study, Glypican-1 was recently projected to be an identifier of cancer exosomes that can detect early pancreatic cancer [42].

A few studies have also been performed for evaluating the role of exosomal microRNAs (miRNAs) in pancreatic cancer. For example, Charrier et al. have shown that connective tissue growth factor (CCN2) and miR-21 are components of a positive feedback loop in pancreatic stellate cells (PSC) during chronic pancreatitis, and are exported by PSC-derived exosomes [39]. Similarly, pancreatic cancer derived exosomes have been shown to regulate the expression of TLR4 in dendritic cells via miR-203 [40].

Some work has also been done toward demonstrating the diagnostic utility of exosomes in pancreatic cancer. For example, it was shown that pancreatic cancer derived exosomes can be used in salivary biomarker development [43]. The analysis of serum exosomal miRNAs has been performed to discover the link between clinicopathologic features of patients with pancreatic adenocarcinoma [44]. More recently, a superior approach involving combined evaluation of a panel of protein and miRNA as serum-exosome biomarkers for pancreatic cancer diagnosis has been shown to have increased sensitivity and specificity [45].

Exosomal content has been linked to pancreatic cancer drug resistance as well. As a proof of concept, an exosomal lipid was shown to induce human pancreatic tumor MiaPaCa-2 cells resistance through the CXCR4-SDF-1a signaling axis [46]. Building on these findings, some exosome based therapeutics has been tested pre-clinically. For example Aspe et al. have clearly demonstrated the enhancement of gemcitabine sensitivity in pancreatic adenocarcinoma by novel exosome-mediated delivery of the Survivin-T34A mutant [47]. In another study, paclitaxel incorporated by mesenchymal stromal cells and released in exosomes was shown to inhibit in vitro tumor growth of pancreatic cancer, and thus making it a new mode of drug delivery [48]. In regards to natural products, Osterman et al., have recently shown that Curcumin modulates pancreatic adenocarcinoma cellderived exosomal function [49]. Collectively, exosomes have been clearly shown to play some important role in the biology of pancreatic cancer. As presented in some of the studies above, there is clear indication that the power of exosomes could be harnessed for

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pancreatic cancer diagnostics as well as for the development of novel therapeutics in the near future.

Conclusion

Vesicular research has gained momentum in the last several years. From 6 papers per year in 2000, the publication list per year stand at more than 663 for 2014 and is currently at 513 for ongoing year of 2015 (PubMed search results per year using the key word Exosomes). These studies have laid the foundations of some novel methodologies in diagnostics against complex cancers especially pancreatic cancer. The exosome based methodologies are touted to be the next generation strategy for the successful delivery of efficacious drugs and their combinations for the successful treatment of pancreatic cancer. Exosomes have also been predicted to be used in the successful delivery of miRNAs for very precise and selective regulation of gene expression in pancreatic tumors for desirable outcome. However, more work is needed before these tiny vesicles can be incorporated into mainstream pancreatic cancer medicine. Given the ongoing intensive activity across a broad spectrum of interdisciplinary fields, it is anticipated that we are quite close to realizing the dream of unveiling the hidden potential of these tiny vesicles to ultimately find the panacea for pancreatic cancer.

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