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Review article

The role of exosomes in the pathogenesis of pancreatic ductal adenocarcinoma

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ABSTRACT

Exosomes are small membrane bound vesicles secreted by cancer cells that have a cytosol rich in proteins and nucleic acids which are capable of modulating the phenotype of neighbouring cells which take them up. In this review we explore the mechanisms through which exosomes are able to impact on the pathogenesis of pancreatic ductal cancer through the modulation of tumour formation and development and exploitation of the tumour microenvironment to modulate both the adaptive and innate immune response. In addition we highlight the potential utility of exosomes not only as biomarkers of disease but also as tools to be used in the therapeutic armamentarium against this disease.

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1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) represents the 10th commonest cancer diagnoses in the UK with 8662 new diagnoses made in 2012. The disease has an extremely poor prognosis with the number of deaths from this disease matching the number of new caseson a 1:1 basis (8773 deaths in 2011) making it the 5th commonest cause of cancer related mortality in the UK (Cancer Research UK, 2014). Surgical resection is the treatment of choice for patients with PDAC with a median survival of 2 years typically being achieved although 80% of patients are unsuitable for surgery at the time of presentation (Chang et al., 2014; Neoptolemos et al., 2010).

For those patients not suitable for surgery systemic chemotherapy is the treatment of choice. Response rates to systemic chemotherapy are poor with the median survival of Gemcitabine treated patients being less than 6 months (Burris et al., 1997). More recently treatment with the triple agent regimen of 5-FU, oxaliplatin and irinotecan (FOLFIRINOX) has been reported to achieve a median survival of just over 11 months although this regimen is associated with significant toxicity and only suitable for patients

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with a good performance status (Conroy et al., 2011). In order to improve these dismal outcomes it is necessary to develop a better understanding of PDAC biology and identify means of exploiting this knowledge for patient benefit.

PDAC is associated with a rich stroma that is typically far more abundant than the epithelial component of the tumour. This stroma contains a wide variety of cell types including pancreatic stellate cells, fibroblasts, myofibroblasts, immune cells, vascular endothelial cells and extracellular matrix which together make up the tumour micro-environment (TME). This TME plays a pivotal role in the regulation of a variety of aspects of tumour behaviour including proliferation, resistance to drug therapy, metastases development and modulation of the immune response to tumour (Feig et al., 2012). Developing a better understanding of how the tumour develops its microenvironment and manipulates it to facilitate these processes may open up novel avenues for the treatment of PDAC.

Exosomes are small membrane bound vesicles typically 40–150 nm in size that are actively secreted by wide variety of cells including cancer cells. Structurally they consist of a lipid bi-layer envelope surrounding a cytosol that is devoid of the usual cellular organelles (*e.g.* mitochondria, ribosomes). This cytosol is rich in proteins (*e.g.* heat shock proteins, cell signalling proteins) and nucleic acids (messenger RNA, micro RNA, DNA) that are capable of biological activity in the neighbouring cells that take them up (Azmi et al., 2013; Kahlert et al., 2014; Mittelbrunn et al., 2011; van den Boorn et al., 2013).

The content of exosomes is not determined randomly but rather is specifically packaged in intracellular endosomes prior to release into the extracellular environment and may, in the case of tumours, be influenced by common genetic mutations such as K-Ras (Cha et al., 2015; Squadrito et al., 2014). Similarly the process of exosome release into the extracellular environment is not a random passive process but rather is tightly regulated by a variety of mechanisms including expression of the Rab family GTPases Rab27a and 27b as well as ceramide, whose production is dependent on neutral sphingomyelinase 2 (Mittelbrunn et al., 2011; Ostrowski et al., 2010).

Upon release into the extracellular environment there is evidence to suggest that exosome interaction with target cells is directed by the expression of membrane proteins such as heparin sulphate proteoglycans and phosphatidylserine receptors (Christianson et al., 2013; Miyanishi et al., 2007). In addition the expression of specific integrins or tetraspanins on the exosomal membrane seems to play a key role in their targeting to either specific organs or cells (Hoshino et al., 2015; Nazarenko et al., 2010). Together this implies that cancer cells are able to release exosomes that are able to selectively target specific stromal cells and manipulate these cells in a manner that favours tumour progression. This communication however is not a one-way phenomenon with cancer cells also taking up exosomes released from stromal cells as well as neighbouring cancer cells (Zhao et al., 2016).

Over the last decade there has been an explosion in exosome related research in virtually all cancer types. This has led to exosomes being implicated in a wide variety of cancer related processes including tumour proliferation; angiogenesis; inhibition of apoptosis; promotion of epithelial to mesenchymal transition; promotion of tumour invasiveness and metastasis formation; development of the metastatic niche; induction of immune tolerance and the development of chemoresistance (Azmi et al., 2013; Yu et al., 2015). This functional role of exosomes in cancer development, as well as their ability to be easily isolated from bodily fluids such as serum, makes them an attractive candidate for biomarker development. In addition, more recently, there has been a growing interest in the therapeutic manipulation of exosomes as a novel approach to cancer treatment.

The aim of this review is to explore the emerging role of exosomes in the development and progression of PDAC and to explore the potential utility of exosomes in both the diagnoses and treatment of this disease.

1.1. Glypican 1 expressing exosomes in PDAC development

GPC1 is a heparin sulphate proteoglycan that is overexpressed in primary pancreatic cancers, predominantly within fibroblasts of the TME, and which is actively secreted by pancreatic cancer cell lines *in vitro* (Kleeff et al., 1998). In 2012 Whipple et al. developed a mouse strain that not only had oncogenic KRAS and loss of INK4A, a combination which leads to the spontaneous development of PDAC, but also had complete loss of GPC1 (Pdx1-CRE; LSL-Kras^{G12D},INK4A$^{LOX/LOX}$; GPC1$^{-/-}$). At 30 days of age only 1 in 10 of the GPC1$^{-/-}$ mice had developed PDAC as compared to 7 out of 10 GPC1$^{+/+}$ mice implying an important role for GPC1 in pancreatic tumourigenesis. Whilst there was no difference in stromal density in the tumours of GPC1$^{-/-}$ as compared to GPC1$^{+/+}$ mice there was significantly less proliferation and angiogenesis present in the tumours of these animals. Similarly tumours from GPC1$^{-/-}$ mice demonstrated a significantly diminished metastatic capacity (Whipple et al., 2012).

In the study by Whipple et al. the impact of GPC1 depletion on tumour behaviour was attributed to decreased responsiveness to heparin-binding growth factors (HBGFs), in particular fibroblast growth factor-2 (FGF-2), for which GPC1 is an essential co-receptor (Berman et al., 1999). Heparin sulphate proteoglycans, such as GPC1, have been demonstrated to be pivotal in the uptake of exosomes and treatment of cells with both unfractionated and low molecular weight heparin can prevent their uptake – an approach that has also been shown to inhibit tumour growth and metastases formation in animal models of pancreatic cancer (Alyahya et al., 2015; Christianson et al., 2013; Sudha et al., 2014) implying that at least some of the effects seen in the GPC1$^{-/-}$ mouse could be due to altered exosome signalling.

In a seminal paper published this year in the journal Nature, Melo et al. described how exosomes expressing the membrane protein GPC1 can be detected in the serum of patients with pancreatic cancer and that these exosomes can be used to distinguish these patients from both healthy volunteers and patients with chronic pancreatitis. Furthermore the level of GPC1 positive exosomes present in the serum correlated positively with disease burden. Using the PKT genetically engineered mouse model of PDAC (Ptf1a$^{cre/+}$; LSL-Kras$^{G12D/+}$; Tgfbr2$^{L/L}$) the authors were able to demonstrate that the proportion of GPC1^{+} exosomes in the blood of these animals increased proportionately with time and their presence pre-dated the development of MRI detectable pancreatic lesions, implying a role for these exosomes in tumour development (Melo et al., 2015). What, if any, the functional role of exosomal GPC1 is on PDAC development remains unclear, certainly it does not appear to play any role in exosome uptake since heparinase treatment of GPC1^{+} exosomes has no impact on their subsequent uptake by cancer cells (Christianson et al., 2013).

Despite the promising evidence presented it seems highly unlikely that exosomal GPC1 will prove to be a specific biomarker for the diagnosis of PDAC. Indeed in the study by Melo et al. they demonstrated that GPC1 positive exosomes were also detected in the serum of patients with breast cancer (Melo et al., 2015). In addition GPC1 has been identified in peptides secreted in the media of cultured colorectal cancer cells (Greening et al., 2013). Nonetheless GPC1 positive exosomes are likely to be at least cancer specific and the presence of this membrane marker may be of value in the isolation of a cancer specific population of exosomes worthy of more detailed study to determine how they contribute to the development of PDAC. In addition GPC1 exosomes seem to be more