Editorial

Exosome targeted therapy—a step in the future

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World-wide 265,000 people are diagnosed with pancreatic cancer each year, of which 74% will die within 1 year. Most pancreatic adenocarcinomas (PDACs) are diagnosed at an advanced stage with 91% of the patients with regional metastatic disease (1). As a result, PDAC has one of the worst prognoses among all cancers with a 5-year survival of less than 5%. It is one of the few cancers still increasing in incidence and by 2030, if current trends continue and without the discovery of effective treatments, PDAC will be the second leading cause of cancer death in the US (2). Despite these alarming statistics, treatments have not advanced for decades. Regardless of refinements in adjuvant and neoadjuvant therapies, surgical resection for the meager 20% patient who qualified remains the only chance for long-term survival. However, surgically resected patients develop recurrence in 80% of cases, and die rapidly of local or distant metastatic disease within about 20 months of initial diagnosis (3). Nevertheless, over 60% of long term survivor patients survive despite having had features of more aggressive disease such as lymph node metastases (N1) and positive resection margins (R1) (3-5). These statistics reveal a large gap in our understanding of the biology of this disease and this has a tremendous impact on the treatment patients receive.

Extracellular vesicles (EVs) are a heterogeneous group of particles released from normal healthy cells and serve a variety of functions ranging from cell signalling to the modulation of immune responses; it is therefore no surprise that cancer cells both inherit this unique communication system and exploit their diverse properties (6). EVs as a group contain particles of varying sizes and are derived from varying cellular compartments, the smaller group of particles i.e., exosomes, range in size from 50–100 nm and are the particles which are garnering a great deal of focus in the scientific literature. Exosomes are generated in the late endosome and contain nucleic acids, lipids, and protein contents along with membrane proteins on their surface which is a direct reflection of their cell of origin. Exosomes are secreted in diverse body fluids and are involved in various biological processes, including the intercellular exchange of regulatory materials, such as miRNA (6,7). In relation to PDAC derived exosomes (PDAC-DEs), there is growing evidence of their central role in the tumour metastatic potential. The integrins at the PDAC-DE surface drive the organotropism displayed by the PDAC-DE (8) which is similar to the localisation of PDAC metastasis [liver, peritoneum, lung, and abdominal lymph nodes (9)]. Once established in the distant site, the PDAC-DE prepare a suitable microenvironmental "niche" for the PDAC cells to migrate, attach and grow (10) possibly explaining why micro-metastases are present in most patients upon diagnosis.

Numerous studies have previously banked on the high frequency of *KRAS* mutation in PDAC and its key role in tumour cellular functions to develop targeted PDAC treatment with mitigated success (11). These studies, including RNA interference-based approach, were faced with the selection of an effective system that would efficiently deliver its content and not be cleared rapidly from the circulation. The exosome homing properties, their membrane composition facilitating endocytosis, the role they assume as intercellular transporters of cellular

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information, coupled with the potential of increased drug stability and the protection against side-effects, have prompted researchers to use them as a delivery system of chemotherapeutic agents (12), miRNA (13,14), and gene therapy (15) to tumour cells. The article published recently in Nature by Kamerkar *et al.* illustrates such utilisation, confirming the exosome potential for a therapy targeting *KRAS* in PDAC mouse models and concluding that they can become the carrier of choice for targeted therapy (16).

Kamerkar *et al.* conducted in parallel a comparison between $CD47^+$ normal foreskin fibroblast culture derived exosomes and liposomes. They first demonstrated that CD47, a transmembrane protein protecting cells against macrophage phagocytosis, exercised the same protective role on exosomes and increased their localisation into the pancreas. Using electroporation, they inserted siRNA or shRNA targeting the *KRAS* G12D mutant. They demonstrated the superior efficacy and specificity of the iExosomes *in vitro* to specifically suppress the activity of the targeted *KRAS*, to impair cell proliferation, and to enhance apoptosis. Used as treatment *in vivo* in nude mice, the repeated injection of iExosomes significantly reduced tumours to nearly undetectable levels and suppressed the tumour growth for 10 days after treatment interruption. However, in the advanced stage the treatment lost its efficacy. In immunocompetent mice, the treatment reduced tumour growth but was not as effective as in the nude mouse model. The authors also identified three possible mechanisms related to iExosome treatment effectiveness *in vivo* over the liposome: the presence of CD47 to elude macrophage clearance, RAS-mediated enhanced macropinocytosis, and the presence of proteins on the exosome surface to increase uptake by tumour cells. Other than increasing survival, the treatment also limited metastasis formation and did not show cytotoxicity. The authors did an impressive and exhaustive study that is worth mentioning on the superiority of exosomal *KRAS* targeted treatment in different mouse models. However, several questions still remain concerning its transfer into human studies.

The use of exosomes as a delivery system is an area of in depth research (17), but the current state of the technology leaves many open questions particularly as to its scalability to a human model and their potential for immunogenic recognition. Kamerkar *et al.* utilize the BJ foreskin fibroblast cell line as the source of the exosomes; this line is unusual since it grows far longer than normal for normal human fibroblasts with up to 72 population doublings (18). The potential for the exosomes from these cells to transmit unwanted cargo is a concern, given the very nature of exosome cargo i.e., RNA and proteins of the producer cell, these could potentially transmit growth promoting cargo. Therefore, the technology needs to develop to ensure that the source cells for exosome based therapy convey no risk. The scalability to the human model may also give rise to manufacturing issues since Kamerkar *et al.* use 10^8 iExosomes per injection into the mice with localized disease. In humans the number required to get a therapeutic effect is likely to increase significantly and may lead to a need for far higher numbers of iExosomes in patients with advanced metastatic disease, this again reflecting back on the source cells used to generate the iExosomes.

The current manuscript by Kamerkar *et al.* also noted a decreased efficacy of the iExosomes in the immunocompetent mouse, the fact that EV's as a group have MHC-I and II (19) may be responsible for their reduced effect in the immunocompetent mouse; with the mice likely mounting responses to the presence of human MHC-I on the iExosomes sourced from the BJ human fibroblasts. The role of immunity to EV's in general can be gleaned from ongoing research in the field of organ donation where acute rejection of allografts may be linked to the presence of donor derived EVs present within the graft itself (20,21). The current predominate bias of the use of human derived exosomes into immune deficient mice models is likely to be a major barrier moving forward to the use of exosomes from a none matched MHC-I human source into an immune competent human.

Through the efforts of the large-scale sequencing projects over the past 6 years, we now understand the genomic alterations contributing to PDAC in unprecedented detail (22-26). These studies have confirmed the initiating role of *KRAS* activation in PDAC with the detection of hotpoint mutations at codons 12, 13, or 61 in over 90% of cases (23) but they have also identified tumours with clonal heterogeneity in *KRAS* mutations (26). Moreover, through multi-omics studies we are also coming to a better understanding of the diverse processes driving pancreatic metastasis (27). The result is that we have a dramatically improved understanding of the somatic genetics of PDAC tumorigenesis and of its complexity. We are now aware of the presence and genetic make-up of tumour sub-clones with different metastatic potential and have identified several PDAC subtypes based on gene expression and prevalent disturbed pathways (22-26). Over the last decade, the avalanche of mutation data for other tumours

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has prompted pharmaceutical companies to develop an armamentarium of agents to attack various protein targets. Today scores of targeted agents (mainly small molecular inhibitors and monoclonal antibodies) are in use, and more are in clinical trials or in the industry's development pipeline. The disappointing fact is that most patients with advanced cancers treated with these new agents recur eventually with metastasis that are resistant to treatment. Will a treatment, even with the use of exosome as a delivery system, using a single target, the oncogenic *KRAS*, be more efficient for PDAC? The complexity of the disease that has been revealed and the heterogeneity of the tumour and even clonality in *KRAS* mutation, question the use of a single approach as treatment. Moreover, as demonstrated in the KRasG12D;Pdx1-Cre (KC) mouse model, *KRAS* activation initiates the formation of precancerous lesions but is not sufficient in 90% of cases to induce transformation and require the inactivation of *TP53*, *SMAD4*, and *CDKN2A* for PDAC to develop (11). This implies that *KRAS* inactivation in a transformed tumour might not be sufficient to induce its complete destruction. This might be one of the reasons why Kamerkar *et al.* failed to totally eradicate the tumours. In all their mouse models, the *KRAS* targeted treatment slowed the tumour growth and prolonged life but was never a cure, whilst also requiring continuous treatment to control tumour growth. Following studies should analyse the effect of combine exosome targeted *KRAS* therapy with conventional therapy or even other targeted therapies. This might be the way to circumvent the weakness of each of these treatments. One such example resides in the key role that the PDAC microenvironment plays in fibrosis, hypoxia, and hypovascularisation (28,29) which hamper the transport of the drugs through the tumour and might affect also exosome transport. A combined treatment that could target PDAC stroma might also be beneficial (30). Other examples of combined targeted therapies could include PARP-inhibition, platinum based chemotherapy and would require that these therapies be personalized based on the make-up of each patient tumour (26).

In this time of broad and deep knowledge one should not ignore what we have learned from previous work. The use of a one-track approach which favours the expansion of aggressive clones resistant to therapy is not a valuable strategy any longer. It might not be practical or possible to use excessive amount of iExosomes but we might be able to design multiple target integrated therapies to induce a chain reaction of tumour destruction that may also harness the patient immune system in the fight.

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