Editorial

Therapeutic targeting of oncogenic KRAS in pancreatic cancer by engineered exosomes

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Exosomes, the smallest (30–150 nm in diameter) subset of extracellular vesicles (EVs) are derived from the endocytic compartment of the parent cell and function as an intercellular communication system (1). The system operates in unicellular as well as multicellular organisms (2) and involves the transfer of signals and/or genetic messages from the parent to recipient cells (3). The mechanisms responsible for the message delivery to recipient cells have been intensively investigated and found to vary from the receptor/ligand-type uptake to macropinocytosis, endocytosis, or phagocytosis (4). The nature of recipient cells seems to determine the mechanism of exosome uptake. Tumor cells, including pancreatic adenocarcinomas, readily uptake exosomes engaging one or more of these mechanisms. Exosomes carry a rich cargo of integrins and opsonins that facilitate their uptake (5). *In vivo*, the time exosomes spend in the circulation is another factor that regulates their uptake. The removal of exosomes from the circulation by cells of the reticuloendothelial system (RES) is likely to be rapid, potentially interfering with the targeted delivery of the exosome cargo to recipient cells.

Exosomes are considered to be promising delivery vehicles for therapeutic agents such as, e.g., RNA interference (RNAi) (6). It appears that exosomes, like liposomes, can be readily loaded with RNAi. Because exosomes carry proteins enhancing their uptake, it was assumed that they would efficiently deliver RNAi to targeted recipient cells. However, enhanced exosome phagocytosis by monocytes/macrophages represents a potential hurdle of rapid exosome clearance from the circulation that might interfere with the intended RNAi delivery to target cells. In a recent paper published in *Nature* (7), Kamerkar and his group report that exosomes derived from fibroblast-like mesenchymal cells carry CD47, an immunoglobulin-like domain-containing molecule that serves as a "don't eat me" signal. The binding of CD47 located on the exosome surface to its receptor, signal regulatory protein alpha (SIRPα), on phagocytic cells inhibits phagocytic functions (8). CD47 is broadly expressed on various types of tumor cells and normal tissue cells, and the antibody-mediated blockade of its tumor-protective functions has been used as therapy in patients with cancer (9). Similar to protecting tumor cells from phagocytosis, CD47 on exosomes contributes to suppression of their clearance and increases the efficiency of targeted content delivery. In a series of elegant experiments, the authors of the *Nature* paper successfully electroporated $CD47^{high}$ exosomes with Alexa Fluor 647 (AF647)-labeled short interfering RNA (siRNA), creating "iExosomes". These exosomes delivered intraperitoneally (IP) to CD57BL/6 or nude mice showed better retention in the circulation and better accumulation in the liver, lung and pancreas than liposomes. Importantly, CD47 knockout (KO) exosomes showed significantly less retention, suggesting that CD47 presence on exosomes limits their clearance by circulating $SIRP\alpha^{+}CD11^{+}$ monocytes. In the presence of blocking anti-CD47 antibody, disrupting the CD47-SIRPα signaling in monocytes, a significant increase of $AF647^{+}CD11b^{+}$ monocytes (i.e.,

monocytes internalizing labeled exosomes) in the circulation was observed. When $CD47^{high}$ iExosomes were injected into mice, a decrease in circulating $AF647^{+}CD11b^{+}$ monocytes was evident, suggesting that exosome escape from clearance by monocytes was partly mediated by the CD45/SIRPα signal.

The objective of the above-described iExosome delivery studies was to pave the way for implementation of RNAi-based therapy targeting oncogenic KRAS in pancreatic ductal adenocarcinoma (PDAC). Earlier studies suggested that oncogenic RAS upregulated macropinocytosis in pancreatic cancer cells facilitating exosome uptake. The investigators electroporated exosomes with siRNA or short hairpin RNA (shRNA) targeting $KRAS^{G12D}$ and showed that these iExosomes were internalized by human PANC-1 cells and significantly decreased $KRAS^{G12D}$ mRNA levels and RAS activity in these cells. This effect was specific, as cells containing wild-type *KRAS* were not altered. In the next series of experiments, mice with luciferase expressing orthotopic PANC-1 tumors were treated with repeated IP injections of iExosomes or iLiposomes (10^8 every other day). Tumors treated with iExosomes were significantly reduced in size relative to controls, and overall survival of mice was significantly improved. iLiposomes were less effective. The anti-tumor efficiency of iExosomes was significantly inhibited by blocking the CD47-SIRPα "don't eat me" signal. Further, CD47 KO iExosomes did not robustly suppress tumor growth or prolong survival. The *in vivo* experiments in several different mouse models of pancreatic cancer confirmed the remarkable therapeutic efficacy of iExosomes that far surpassed that of liposomes. In additional *in vivo* experiments, the authors of the *Nature* paper also showed that iExosomes generated from mouse or human sources delivered to animals bearing advanced pancreatic tumors reduced tumor burden, decreased pancreatic desmoplasia, suppressed proliferation and enhanced apoptosis of cancer cells, reduced ERK phosphorylation, AKT phosphorylation and RAS levels as well as oncogenic $KRAS^{G12D}$ expression with increased animal survival.

The reported significant advantages of therapy with engineered iExosomes targeting oncogenic KRAS in advanced PDAC in mice are especially impressive in light of the evidence that the RAS pathway is poorly druggable (10). What accounts for this impressive therapeutic potential of iExosomes that extended to advanced metastatic disease in this study? And why is iExosome therapeutically superior to iLiposome? The authors suggest that a longer retention of iExosomes in the circulation due to the CD47 content, the RAS-mediated enhanced micropinocytosis in tumor cells, and the presence in the iExosome cargo of proteins facilitating uptake by recipient tumor cells might have accounted for the observed improved therapeutic efficacy of iExosomes *vs.* iLiposomes. To test the first possibility, the authors could have further evaluated the role of CD47 in this process by decorating liposomes with CD47 and following their delivery and therapeutic effects. However, the clue to superior therapeutic effects of iExosomes may be more complex. While CD47 is the best known "don't eat me" protein, there are bound to be others on the surface of various cells and carried by exosomes. The likely presence in the circulation of monocyte-derived $SIRP\alpha^{+}$ exosomes could antagonize the "don't eat me" exosome-to-monocyte signaling (11). Further, there are numerous known "pro-eat me" molecules, including calreticulin or phosphatidyl serine or other pro-apoptotic proteins on tumor cells (12) and on exosomes these cells produce. Hence, the balance of exosomal "pro-eat me" *vs.* "don't eat me" exosome components will surely impact on iExosomes retention in the circulation *vs.* their uptake by recipient cell. The exosome content of adhesion molecules is rich and varied as is the membrane content of specific ligands and receptors they carry, potentially resulting in differences in the exosome uptake by recipient cells (13). The simultaneous delivery by exosomes of many cognate receptor/ligands to recipient cell surface and the resulting signaling represents a powerful and rapid message delivery system that may outperform the other uptake mechanisms. Hence, iExosomes naturally endowed with the roster of specific signaling proteins are likely to deliver more effective messages than synthetic liposomes. Finally, an exosome carries numerous biologically active molecules in its cargo that upon uptake by a recipient cell are necessary to facilitate message translation, which liposomes may or may not be able to do. Also, it is important to remember that cells of the RES may be able to discriminate between biologically-active exosomes and decorated liposomes, differentially regulating their retention in the circulation. This regulation could be altered in advanced neoplasia, where the RES is stressed and the clearance mechanisms are partially overextended.

The *Nature* study convincingly shows that therapy of PDAC with engineered iExosomes targeting oncogenic *KRAS* was effective in reducing tumor growth and was superior to therapy with similarly administered liposomes.

It emphasizes the promising potential of iExosomes in cancer therapy. It comes short, however, of adequately explaining the reasons for the observed therapeutic efficacy of iExosomes. This, of course, is the key question that needs to be addressed prior to further translation of iExosome-based cancer therapy to the clinical setting.

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