Editorial Commentary

Extracellular vesicles show promise for cancer theranostics

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Comment on: Jc Bose R, Uday Kumar S, Zeng Y, *et al.* Tumor Cell-Derived Extracellular Vesicle-Coated Nanocarriers: An Efficient Theranostic Platform for the Cancer-Specific Delivery of Anti-miR-21 and Imaging Agents. ACS Nano 2018;12:10817-32.

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Extracellular vesicles (EVs) are naturally occurring small membrane vesicles (30–150 nm), protected by a lipid bilayer, that originate from endosomes and are formed through the fusion of the plasma membrane with multivesicular endosomes (MVEs), and subsequently exocytosed (1). The roles of EVs are numerous and could be important in cancer biology and disease (2). A recent research topic highlights extracellular vesicles addressing the processes that they regulate in cancer and their potential therapeutic value. The manuscript entitled "Tumor Cell-Derived Extracellular Vesicle-Coated Nanocarriers: An Efficient Theranostic Platform for Cancer-Specific Delivery of AntimiR-21 and Imaging Agents" published in ACSNano by Rajendran Jc Bose *et al.* investigated the delivery of antimiR-21 by tumor extracellular vesicles (TEV) and the attenuation of DOX resistance in breast cancer. Importantly, the authors demonstrated that TEVs may act as a biomimetic source for the activation of gold-iron oxide nanoparticles and observable nanotheranostic characteristics by TEV-gold-iron oxide nanoparticles *in vitro*.

To provide readers with some background context in the field of EV-mediated processes in cancer, we first present several aspects of EVs, from functions in cancer development, roles as potential biomarkers in tumor diagnosis and prognosis and potential roles as delivery vehicles in cancer tumor therapy.

EVs can be found in all cell types including dendritic cells (DCs) (3), T cells (4), B cells (5), neurons (6), and tumor cells (7-9). EVs have also been found in human biological fluids, such as blood and urine (10). Extracellular vesicles are one possible mechanism responsible for the connection between cells of the tumor & its microenvironment (11). The content of the extracellular vesicles depend on the donor cell type they derive from and consist of a plethora of molecules including RNAs [microRNAs (miRNAs) and mRNAs], proteins, signaling peptides, and lipids (12). In addition, EVs can also transfer DNA, thus modifying gene expression in recipient cells and further extending EVs interactions. Moreover, they are involved in local and systemic cell communication (13) and directional transportation of cargoes to specific locations. Different cells derive extracellular vesicles with unique cargoes, commonly miRNAs or mRNA, which may be used as molecular markers in tumor diagnosis, e.g., potential determining the specific cancer subtype.

miRNAs are small non-coding RNAs that control gene expression at a post-transcriptionally and have important functions in tumorigenesis (14). miRNAs are known to be dysregulated in many types of cancer and specific miRNA expression patterns characterize normal versus tumor tissue or differentiated versus poorly differentiated tumors. Thus, miRNAs hold putative value in cancer patient diagnostics and prognostics. Upregulated miRNAs in cancer cells promote carcinogenesis by attenuating tumor suppressor genes, and are known as oncomiRs (oncogenic miRNAs) (15). On the other hand, miRNAs with low expression levels in cancer cells that typically halt cancer progression by suppression of the expression of oncogenes,

are called tumor suppressor microRNAs (16). Silencing highly expressed miRNA with anti-miRNAs (antimiRs or antagomirs) or substituting tumor suppressor miRNAs with miRNA mimetics has been demonstrated as a valuable experimental strategy for the treatment of cancer. However, delivery of miRNA-based therapeutics is challenging (17) and extracellular vesicles have been recently explored as a mechanism for RNA therapy (18).

In a recent review (19) focused on the role of TEV as a tumor therapy, the authors presented evidence implying that ncRNAs can modulate gene expression locally and systemically. In another study, after delivery of mouse exosomal RNA to human cells, different mouse proteins were seen in the recipient cells, confirming that delivery of exosomal mRNA can be translated after introduction to a different cell. This showed that exosomes consisting of mRNA and miRNA, can be transferred to another cell, and can be active in a new location.

Earlier research demonstrated that microRNA-21 (miR-21) is highly-expressed in esophageal tumors and in their TEV's. One study analyzed the action of exosome-miR-21 association in the advancement in esophageal cancer (20). The study discovered that exosomes are absorbed from the outside of the cell into the cytoplasm. The exosomes labeled with Cy3-miR-21 mimics were delivered to new cells through a neutral sphingomyelinase 2 (nSMase2)-dependent technique. High levels of miR-21 from contributor cells dramatically improved the migration and invasion of recipient cells through targeting of programmed cell death 4 (PCDC4) and stimulating the c-Jun N-terminal kinase (JNK) pathway. In another study, a cross-comparison analysis of miRNA-21 and -10b (both have been shown to be involved in breast cancer initiation and metastasis) and mRNA expression profiles of normal cells MCF10A cells that were exposed to MDA-MB-231 cancer exosomes revealed a correlation between the up-regulated miRNA-21 and -10b and a down-regulation of their mRNA targets, PTEN and HOXD10. Moreover, immunoblots of PTEN and HOXD10 confirmed they were silenced in MCF10A cells exposed to cancer exosomes (2).

In this current manuscript by Rajendran Jc Bose *et al.*, demonstrated a simplicity in the processing of cell derived materials and packaging of miRNAs in TEVs, and that gold-iron oxide nanoparticle activation offers a new platform for cancer therapeutics and imaging. The authors emphasise the advantages of cell-derived vesicles such as biocompatibility, minimal toxicity, safety, simplicity of construction, loading of diverse groups of agents, and crucially promotion of tissue-specific delivery. They initially investigated cellular internalization and release of intracellular Cy5-antimiRNA-21 from TEVs-Cy5-antimiRNA-21 in homologous tumor cells and then in another type of tumor cell and found that the Cy5-fluorescence signal peak gets higher with time of incubation and concentration of TEVs. This was also confirmed by measuring high levels of antimiR-21 using real-time PCR. The effect of delivered antimiR-21 was further confirmed by quantification of miR-21 downstream target gene levels—the levels of PTEN and PDCD4 were dramatically increased. Moreover, the authors showed that the simultaneous treatment with TEV antimiRNA-21 and low-dose DOX, to 4T1 tumor cells resulted in a spindle-like configuration, cell detachment and apoptotic death. This which was not observed in cells treated with TEVantimiR-21 alone nor DOX alone. In addition, the authors evaluated the use of TEV with gold-iron-oxide nanoparticles (GIONs) which act as a photosensitizer for photothermal therapy (PTT). The outcome of the activity of TEVs-antimiRNA-21 on GIONs was enhanced cytotoxicity, whilst conversely cells not treated preserved their activity. *In vivo* biodistribution indicated high accumulation in tumors of TEV-GION or TEVGION-antimiRNA-21 even after 12 days of repeated post-injection. AntimiRNA-21 distribution in tumors and primary organs was evaluated by real time PCR with a high level of GION-antimiRNA-21 observed in the 4T1 tumors versus different organs. Moreover, antimiRNA-21 was dramatically increased also in the liver, lung, and brain. TEVGION- antimiRNA-21 was also further tested as a diagnostic tool via MRI.

In conclusion, TEV-mediated delivery of miRNA is a promising tool for cancer theranostics. It may function as a biomarker and as delivery vehicle of anticancer drugs. However, the isolation and processing of EVs is still not consistently reproducible and further optimization is still required. Ongoing studies regarding the cargo contents of TEV are required and will help to identify relevant biomarkers and targeting residues. Exciting times are ahead of us as EVs make progress towards being successful delivery agents for cancer therapy.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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