ACS SENSORS

pubs.acs.org/acssensors

Review

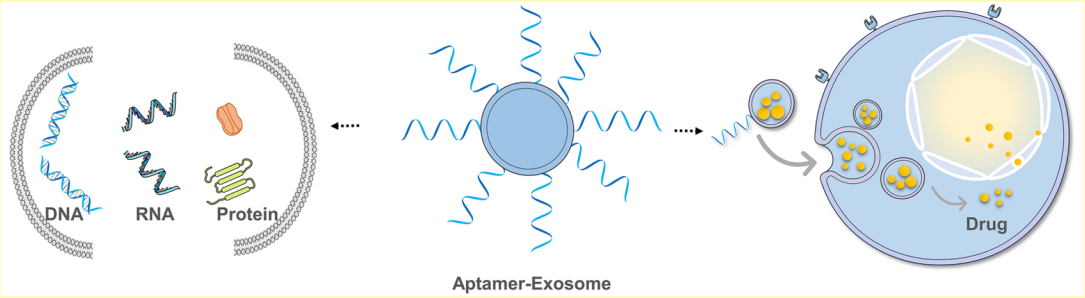
Aptamer–Exosomes for Tumor Theranostics

Kezhen Yi,[#] Yuan Rong,[#] Lanxiang Huang, Xuan Tang, Qian Zhang, Wei Wang, Jianyuan Wu,* and Fubing Wang*

Cite This: *ACS Sens.* 2021, 6, 1418–1429

Read Online

ACCESS | Metrics & More | Article Recommendations



ABSTRACT: As carriers of biomolecules (proteins, nucleic acids, and lipids) from parent cells, exosomes play a significant role in physiology and pathology. In any diseased state, the morphology of the released exosomes remained similar. The contents of exosomes change depending on the disease or its stage; thus, exosomes are generally considered as a "source of biomarkers". Therefore, they are considered promising biomarkers for the diagnosis and prognosis of tumors. As natural delivery vehicles, exosomes can protect their cargo from immune clearance and deliver them to other cells through membrane fusion. After being genetically edited at the cell or exosome level, exosomes can be used for treatment with aptamers. Aptamers are short stretches of oligonucleotide sequences or short polypeptides that have been selected in vitro or in vivo, and have a wide range of targets and show excellent binding affinity and specificity. Aptamers have been widely used as molecular probes, and the combination of aptamers with exosomes has become a new direction for exosome-related research and therapeutic development. Here, we summarized various applications of exosomes and aptamers in cancer research, and further analyzed their combination as an "aptamer–exosome". Finally, we propose future directions for the aptamer–exosome in the precise diagnosis or personalized treatment of cancer.

KEYWORDS: *aptamer, exosome, biosensor, microfluidic chip, SELEX, precise diagnosis, personalized treatment, tumor theranostics*

Extracellular vesicles include exosomes, microvesicles, and apoptotic bodies, among which exosomes are 40–200 nm in size.[1,2] Exosomes are vehicles for shuttling genetic and biological information and mediate intercellular communication.[3] Previous studies have found that exosomes play an important role in cancer progression and metastasis,[4,5] suggesting that exosomes are potential tools for cancer diagnosis and treatment.[6,7] In addition, exosomes acquire molecular information from parental cells,[8] which may provide us with an ideal target to view and analyze parental tumor cells without tissue biopsy. The contents of exosomes isolated from different body fluids can be used as biomarkers for cancer diagnosis, prognosis, and predictive biomarkers for response to therapeutics.[9,10] Currently, diagnostic strategies for exosomes focus on detecting or monitoring the concentration[11] of nucleic acids[12−14] and proteins in exosomes.[15] Exosomes are a unique tumor diagnosis platform with rich information and diverse biomarkers. In addition, exosomes exhibit great potential for tumor-targeted therapy.[16] To date, exosomes have been developed as carriers of various therapeutic and diagnostic agents, such as small-molecule chemotherapeutic agents, anti-inflammatory agents, RNA, and proteins.[17] This is due to their intrinsic advantages over conventional synthetic carriers, including their nanometer-level size, excellent carrier properties, and hypo-immunogenicity.

Aptamers are a family of single-stranded nucleic acids isolated from in vitro selective repeats, called systematic evolution of ligands by exponential enrichment (SELEX).[18] They offer unique bioanalytical functions, such as high sensitivity and specificity, resistance to degradation, cost effectiveness, ease of synthesis, and stability under non-

Received: October 26, 2020
Accepted: February 10, 2021
Published: March 23, 2021

https://dx.doi.org/10.1021/acssensors.0c02237
ACS Sens. 2021, 6, 1418–1429

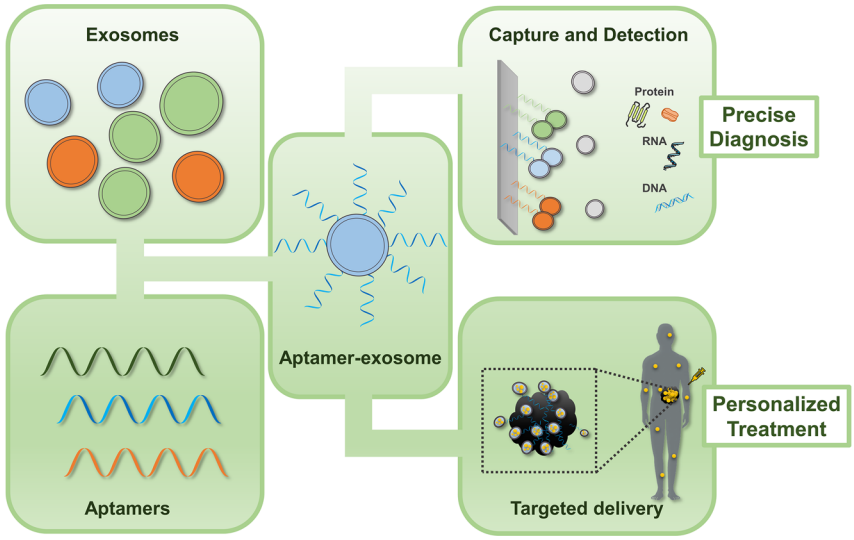


Figure 1. Schematic illustration of aptamer−exosomes for precision medicine and personalized medicine in cancer. The diverse sources of exosomes and their rich contents not only provide us with a mirror reflecting tumor information, but also provide us with various and flexible choices of modifications for drug delivery. With the help of aptamers, the applications of exosomes are greatly enhanced for the diagnosis and treatment in cancer.

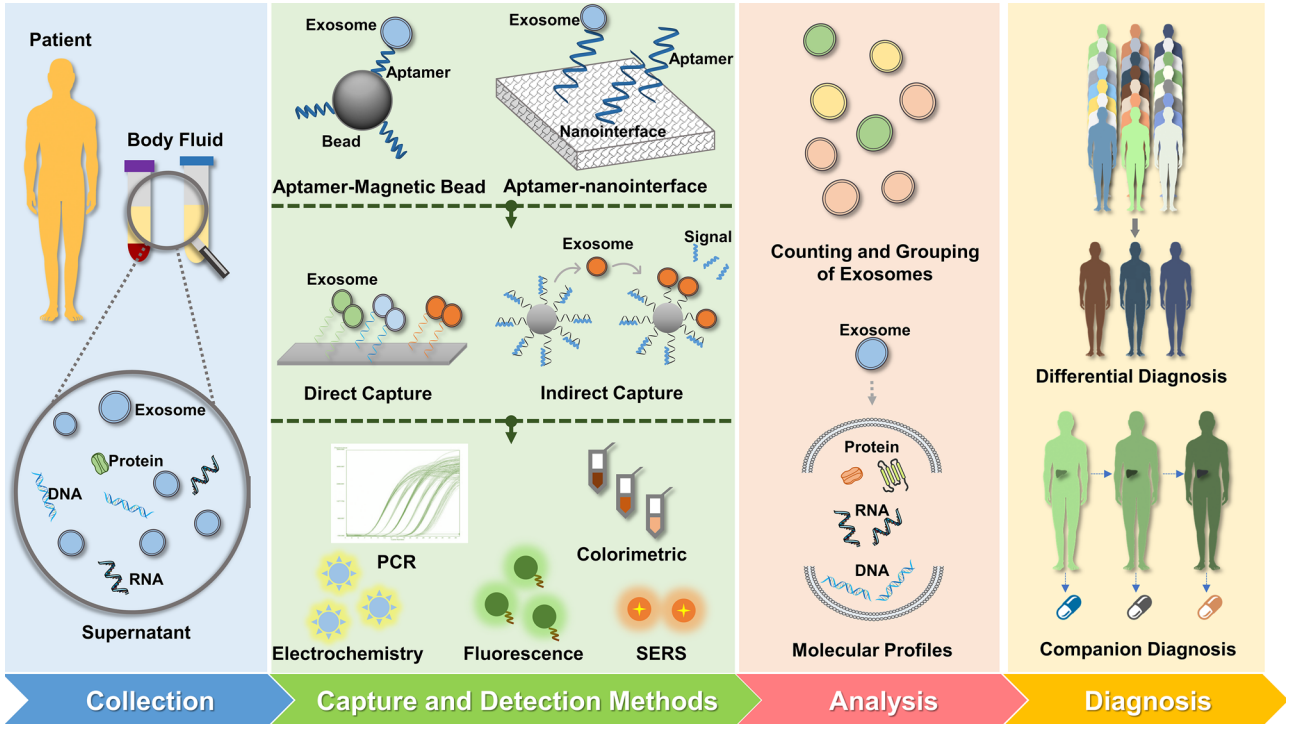


Figure 2. Schematic illustration of the aptamer−exosome applications in cancer diagnosis. The process generally includes collection, capture, detection, and analysis. Exosomes are generally collected from various body fluids according to the characteristics of different tumors. Capture methods can be divided into magnetic and nonmagnetic nanomaterials modified by aptamers. The object of detection can be exosomal molecules or changes in specific components of the aptamer detection system caused by exosomes. Qualitative or quantitative analysis is performed according to the detection requirements. Aptamer−exosome detection system can be used for differential diagnosis and companion diagnosis of diseases.

physiological conditions. Their targets range from a single ion to the entire cell; by modulating the SELEX technique, an aptamer can even target cells at different stages. As far as the sensing design for capture detection is concerned, aptamer platforms are not limited to molecular recognition for specific binding because of their nucleic acid nature, but are also applicable to ultrasensitive and reversible detection based on nucleic acid amplification.[19,20] In addition to making a large impact in cancer diagnosis, aptamers have also made surprising progress in cancer treatment. Aptamers can serve as tumor suppressors[21] and can also be used as carriers for delivering therapeutic drugs to target cells or tissues.[22] In exosome-targeted therapy, aptamers can enhance the targeting capacity of exosomes without causing an immune response.[23]

In summary, exosomes have been widely used for the diagnosis and treatment of tumors. In diagnostics, the diverse

https://dx.doi.org/10.1021/acssensors.0c02237
ACS Sens. 2021, 6, 1418−1429

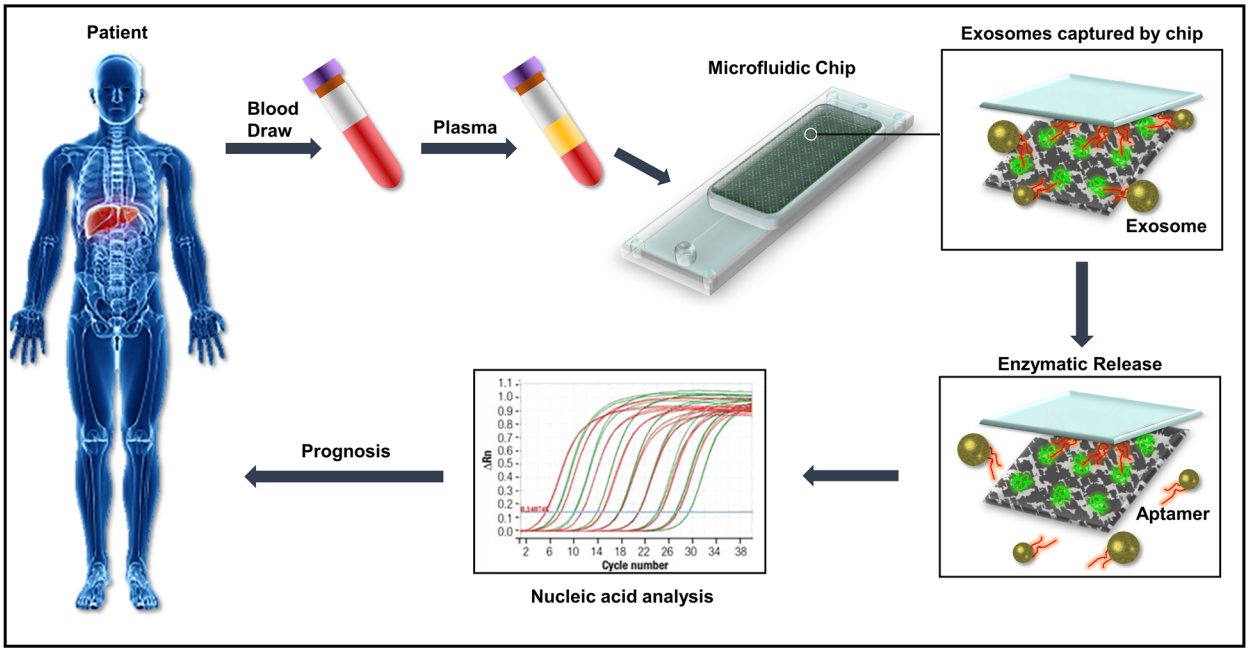


Figure 3. Schematic illustration of a microfluidic chip for capturing exosomes in tumor research. In our previous work, we developed a microfluidic chip based on streptomycin−avidin aptamer capture exosomes. We modified streptomycin on the base of the microfluidic chip, and selected different subpopulations of exosomes based on different and biotinylated aptamers, and analyzed their nucleic acids or proteins to prompt the patient's prognosis.

biological information in exosomes can complement tumor diagnosis, and the abundant target repertoire of exosomes may facilitate the sorting of various subpopulations of exosomes. In therapy, exosomes of different cellular origins provide different biological functions, and can undergo different forms of engineering modifications to target desired cell populations after incorporating aptamers. The application of aptamers improves the accuracy of exosome capture and its capacity to target tumor tissues. Thus, combination of an exosome and aptamer as an aptamer−exosome could unify their advantages for applications in both noninvasive diagnostics and precision tumor therapy (Figure 1).

APTAMER−EXOSOMES FOR CANCER DIAGNOSIS

Exosomes Carry Rich Biological Information from Cancer Cells Facilitating Accurate Diagnosis. The rationale of exosomes as a biomarker for cancer is that the molecular cargo they carry can be considered a molecular fingerprint of the original cancer cells.[24−27] The number of exosomes can be used to determine tumor development. Liu et al. used an immunosorbent assay for the digital identification of target exosomes using droplet microfluidic technology, which has the potential for early diagnosis of breast cancer. In addition, exosomal proteins are important in tumor diagnosis. Melo et al. used exosomal glypican-1 (GPC1) to diagnose pancreatic cancer and distinguish between early and advanced stages of pancreatic cancer.[28,29] GPC1 is a specific exosomal protein screened in pancreatic cancer based on mass spectrometry. In addition, exosomal protein arrays are available for cancer diagnosis. Jakobsen et al. found that patients with non-small cell lung cancer could be distinguished from healthy controls by extracellular vesicle array.[30] Moreover, exosomal nucleic acids are commonly used in tumor diagnosis. MicroRNA expression profiling of eight tumor-specific miRNAs in EpCAM-positive exosomes has been used to distinguish malignant tumors from benign diseases.[31] Exosomal lipids are also used as diagnostic tools. A study showed that the levels of several lipids in urinary exosomes of prostate cancer patients are significantly different from those found in healthy individuals, suggesting that specific molecular lipid species in urinary exosomes could be biomarkers for prostate cancer.[32] In addition to being used to diagnose tumors, exosomes can also be used for tumor grading, monitoring, and prognosis. McKiernan et al. applied the gene expression of urinary exosomes to distinguish high-grade cancer from low-grade cancer and benign disease.[9] In this study, urine exocytosis gene expression was associated with higher grades of prostate cancer in patients with elevated PSA levels, which could reduce unnecessary biopsy collections. Zhan et al. found that upregulation of exosomal lncRNA PCAT-1 and MALAT1 was associated with poor recurrence-free survival in non-muscle invasive bladder cancer patients, and exosomal PCAT-1 overexpression was an independent prognostic factor for this cancer.[33] Exosomes have also been used to predict the effectiveness of immunotherapy. Chen et al. reported that the increase in exosomal PD-L1 stratifies patients without drug response and provides a basis for the application of exosomal PD-L1 as a predictor of anti-PD-1 therapy.[15] Ongoing research from our group also established diagnostic profiles of noncoding RNA in plasma exosomes for liver cancer, bladder cancer, and lung cancer, as well as a diagnostic profile of noncoding RNA in urinary exosomes for bladder cancer. In addition, multiparametric analysis of exosomes is also a developing direction that provides more detailed guidance for tumor diagnosis, especially for prognosis prediction.[34−36] The emerging technologies in these studies provide a more comprehensive analysis of exosomes. In summary, the above examples demonstrate that exosomes can provide an excellent platform for tumor diagnosis.

https://dx.doi.org/10.1021/acssensors.0c02237
ACS Sens. 2021, 6, 1418−1429

Aptamers Provide Various Targets for Capturing Different Types of Exosomes. To date, studies have identified hundreds of targets for aptamers, including ions (such as K^+),[37] small organic compounds (such as AFP),[38] and more complex targets (such as whole living cells).[39−41] The aptamers produced by cell-SELEX can recognize cell surface biomarkers without knowing the specific characteristics of the molecular marker.[42,43] Many tumor proteins are available. Dong et al. used a PSMA aptamer to capture prostate cancer exosomes and established a dual-signal amplification system for exosome detection in a complex environment.[44] In addition to pan-cancer biomarkers, cell-SELEX technology provides tumor cell-specific biomarkers. For example, a study by our group demonstrated that LY-1 is a promising molecular probe that recognizes metastatic HCC cells.[45] There are also genes that target exosomes. Zhang et al. used the CD63 aptamer to capture exosomes and found that the number of MUC1-positive exosomes can be used to distinguish cancer patients from healthy individuals.[46] In summary, the target molecules commonly used in tumor exosome capture by aptamers can be divided into two categories: targeting exosome markers and targeting specific tumor markers. The former capture method obtains total exosomes, which contain information on both tumor and nontumor cells. The latter captures a specific subgroup of tumor-derived exosomes, which may not accurately represent comprehensive information on the tumor. To resolve these limitations, specific markers of tumor-derived exosomes need to be explored. Melo et al. determined that GPC1 was specifically overexpressed in cancer cell-derived exosomes.[28] Instead of using tumor markers as markers of tumor-derived exosomes, researchers are improving their methods by obtaining markers of tumor-derived exosomes.

Although similar detection methods can be developed using antibodies, aptamers have irreplaceable advantages in tumor diagnosis. The most important advantage is that aptamers have a wider range of targets than antibodies. While antibodies can only target known targets, aptamers can bind to unknown targets. In particular, we do not need to determine the specific traits of the target when using aptamers, and can still obtain the population represented by the target. This is because, among aptamer screening techniques, there is a special technique called cell-SELEX that can acquire aptamers with high specificity when the target is unknown.[47−49] However, the constitution of the cell surface is quite complex, so the reverse selection step for nontarget cells needs to be increased; this makes the aptamer screening process more tedious. The steps for identifying aptamer-targeted membrane proteins include extraction of aptamer membrane proteins, identification by SDS-PAGE, protein sequencing, and validation.[50] Thus, cell-SELEX can be used to discover novel cancer cell biomarkers. The next step in the development of aptamer targets is the utilization of tumor-derived exosomes.

Aptamers for Detection of Exosomes. A variety of aptamer-based bioassays for exosomes have been developed. In general, current strategies for using aptamers to detect exosomes in tumor patients include the following steps: first, the body fluids of patients (such as serum or urine) are collected; second, aptamer-modified magnetic beads or nanomaterials are used to capture exosomes. Third, the captured exosomes are analyzed, with analysis including the number of exosomes, exosomal protein, DNA, and RNA profiling; and finally, the diagnosis, prognosis, and treatment guidance of cancer were conducted (Figure 2).

Aptamer−exosomes have been used for tumor diagnosis. Some systems can capture exosomes directly; Zhang et al. were able to rapidly capture exosomes in plasma using a CD63 aptamer, and then used complementary DNA sequences to disrupt aptamers without any damage to exosomes.[46] Therefore, the number of exosomes and complete exosomes can be obtained at the same time. In addition to directly analyzing captured exosomes, the nucleic acid properties of aptamers can also be utilized to convert exosomal information into other signals. Li et al. combined magnetic materials, PSMA aptamers, and the complementary DNA sequences of PSMA aptamers to form a detection system. Once exosomes were captured, the complementary DNA sequence of the aptamer was released, and the amount of DNA released could represent the number of exosomes.[20] In another study, the researchers labeled CD63 aptamers and EpCAM aptamers with different fluorescent dyes as probes and quenched the fluorescence with graphene oxide. In the presence of exosomes, both fluorescent signals are recovered simultaneously. In addition, with the help of the DNase I enzyme, the aptamer on the surface of the exosome is digested, allowing the exosome to interact with more fluorescent aptamer probes, and the amplified fluorescent signal can be detected.[51]

Because aptamers are mainly nucleic acids, they show greater flexibility when designing different sensing formats. Aptamer sensors based on target-induced structural changes, ultrasensitive aptamer sensors based on polymerase and/or nuclease coupling, and microarray/next-generation sequencing aptamer sensors for multivariate analysis have been developed. For example, a detection system based on a surface plasmon resonance (SPR) strategy can amplify exosome signals in body fluids with detection limits as low as 1.00×10^5 particles/mL.[52−54] More commonly, the fluorescence signal-based aptamer−exosome detection system has superior specificity and sensitivity, and the detection limit can reach 30 particles/μL.[55−60] In addition, there are detection systems based on electrochemistry[61] or colorimetric methods.[62] Notably, an aptamer sensor based on ultrasensitive hybridization chain reaction-amplified CRISPR-Cas12a for extracellular vesicle surface protein quantification reached a detection limit as low as 10^2 particles/μL.[63] One study used aptamer-coated lipoplexes to obtain fingerprints of various types of cell-derived exosomes through personalized aptamer cluster selection.[64] Among these existing applications, the most prominent one is to rely on a microfluidic chip for convenient trace detection. In one study, researchers first used fluorescently labeled nucleic acids to specifically recognize tumor-associated membrane proteins on the surface of exosomes, and laser-irradiated microfluidic chips generated temperature gradient-induced thermophoresis, allowing exosomes to rapidly converge to the center of the chip. After exosome converge, the signal of the aptamer is read using a fluorescence microscope to obtain exosome membrane proteomics information. Finally, the study combined machine learning algorithms to use exosome membrane proteomics information for cancer diagnosis, early classification, and recurrence monitoring.[65] Different chip designs may provide diverse tumor point-of-care testing for different needs (Figure 3).

In tumor diagnosis, we believe that the technological progress of aptamers will advance tumor exosome studies and improve our understanding of exosomes.

https://dx.doi.org/10.1021/acssensors.0c02237
ACS Sens. 2021, 6, 1418−1429

APTAMER–EXOSOMES FOR TUMOR TARGET TREATMENT

Exosomes are Flexible Bioengineering Platforms. Exosomes secreted from different cells have different molecules and may therefore have different biological functions.[66−69] During targeted therapy of tumors, exosomes targeting tumor cells can be used to directly kill or inhibit tumors. Alternatively, exosomes targeting immune cells can be prepared, and the tumor can be killed or inhibited by the exosome-induced immune system. For example, after being stimulated by tumor peptides, dendritic cells secrete exosomes that can stimulate specific cytotoxic T lymphocytes in the body, thus eliminating or inhibiting the tumor.[70] Kamerkar et al. used electroporation to load RNA drugs that are specific for oncogenic KRAS into exosomes secreted by fibroblast-like mesenchymal cells, inhibiting cancer and increasing the overall survival rate.[71] It was also demonstrated that CD47 on exosomes can inhibit monocyte clearance. Wang et al. used an excess of natural killer (NK) cell-derived exosomes and biomimetic core−shell nanoparticles for tumor-targeted therapy.[72] In addition to exosomes derived from these naturally occurring cells, exosomes from engineered cells are also useful. The membrane of exosomes released by chimeric antigen receptor (CAR)-T cells carried CAR, which effectively inhibited tumor growth. More importantly, exosomes derived from CAR-T cells are much safer than CAR-T cells.[73]

The low immunogenicity and homing ability of exosomes also improve targeted drug delivery.[71,74] Moreover, compared to other forms of drugs, exosomes can enter specific cells and act on sites inside the cell;[75] thus, the off-target effects are greatly reduced. Melzer et al. found that the effective concentration of paclitaxel in mesenchymal stem cell (MSC)-derived exosomes was lower than that of paclitaxel alone, but the cytotoxicity was higher. These results indicate that exosomes can increase the effectiveness of drug delivery.[76] To date, exosome-based tumor treatments can be categorized into the following three types: (1) selecting a specific cell to produce natural exosomes with specific functions; (2) after bioengineering the cells, the exosomes will contain the expected molecules for better targeting or contain higher levels of active molecules for cancer killing; and (3) modifying exosomes for specific functions; and Yang et al. used cellular nanoporation to combine the two steps of purification and drug loading into one step, so that the exosomes loaded with the target mRNA secreted by the cells improved their yield, targeting, and efficacy.[77] It is more common to genetically modify donor cells to obtain exosomes with specific molecules. Limoni et al. transduced cells with a lentiviral vector carrying a chimeric gene. Stable cells expressing the fusion protein were selected, and exosomes produced by these cells were isolated from the culture medium.[78] Therefore, both the cells and the exosomes were edited for improved efficacy.

In summary, exosomes can be obtained from a wide range of sources, including tumor cells, MSCs, NK cells, and CAR-T cells. Cells can be chemically or genetically edited to secrete exosomes as needed. These allow exosomes to act as self-contained drug modification platforms for tumor treatment.

Aptamers Facilitate Targeted Delivery of Exosomes. Aptamers have been used in a large number of applications for cancer treatment, including serving as anticancer drugs[79−83] or participating in the construction of nanotargeted drug particles.[84] Cancer treatment is very complicated in practical

Table 1. Examples of Aptamer–Exosomes for Cancer Therapy

no.	aptamer–exosomes: name of aptamer	aptamer–exosomes: source of exosomes	drug	tumor	cancer therapy application	remarks	ref.
1	sgc8 (PTK7)	Immature dendritic cells	Dox	Leukemia	Chemotherapy is specifically delivered to target cancer cells.	Compared with free Exos, the cellular uptake mechanism of Exos with targeting ligands is different.	94
2	AS1411 (Nucleolin)	Dendritic cells	paclitaxel	Breast cancer	Potential applications are obtaining exosomes from patients' autologous cells.	The production efficiency and rate of exosomes produced by squeezing cells are higher.	102
3	A9g (PSMA)	HEK293T cell	survivin siRNA	Prostate cancer	siRNA was specifically delivered to tumors and effectively blocked tumor growth.	The orientation of RNA nanoparticles is used to control the loading of siRNA and miRNA or display on the surface of exosomes to achieve targeting function.	103
4	anti-EGFR DNA aptamer	A549 cell	—	Lung adenocarcinoma	Using aptamer-functionalized nanoparticles to excrete carcinogenic exosomes in the blood into the small intestine	A special example of exosomes as therapeutic targets. The binding of Aptamer to exosomes is actually accomplished in vivo.	104

https://dx.doi.org/10.1021/acssensors.0c02237
ACS Sens. 2021, 6, 1418−1429

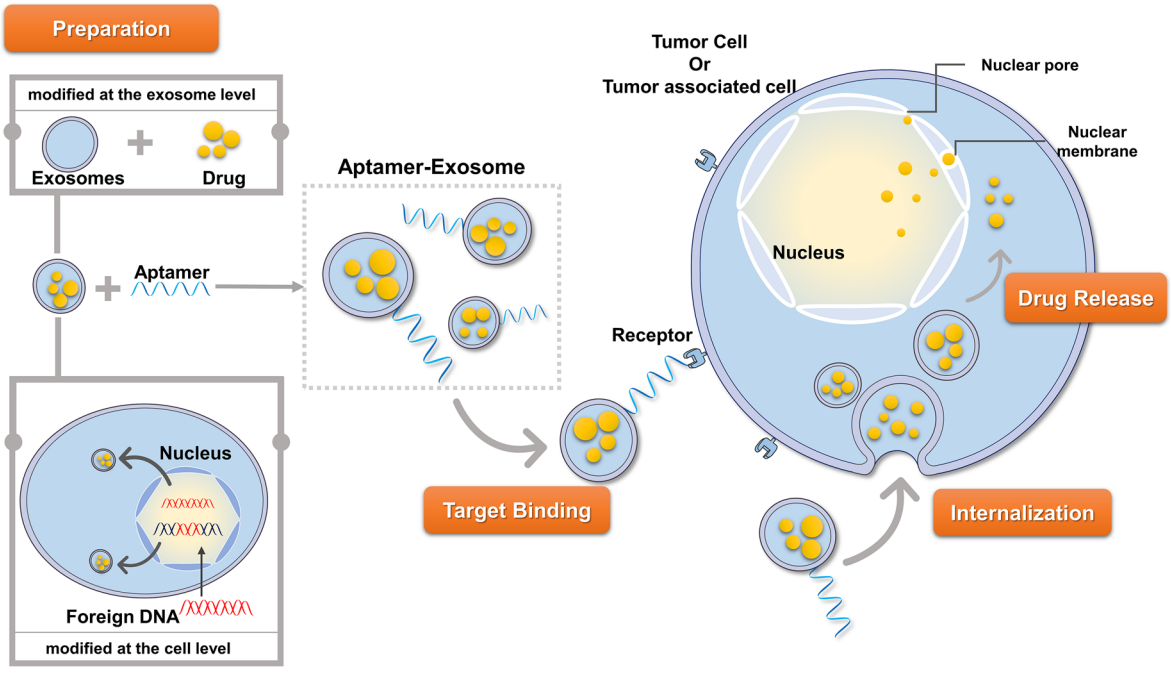


Figure 4. Schematic illustration of the aptamer−exosome applications in tumor treatment. During the preparation process, researchers can modify the cells to produce the required exosomes or modify secreted exosomes. After exosomes are obtained, they can be combined with aptamers to enhance their targeting. Aptamer−exosomes can deliver drug to targeted cells to achieve therapeutic effects.

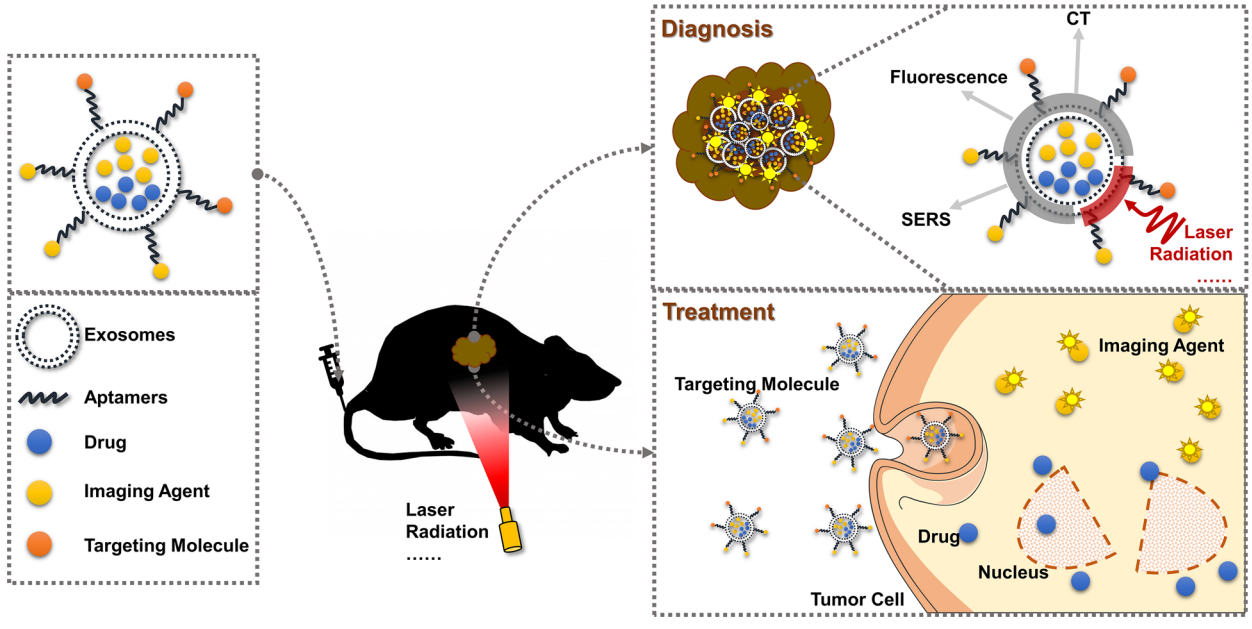


Figure 5. Schematic illustration of the aptamer−exosome applications in tumor theranostics. Theranostic nanoparticles can have both diagnostic and therapeutic effects.

applications.[85] It requires a comprehensive understanding of the integrity and heterogeneity of cancer tissues,[86,87] the characteristics of the cancer microenvironment,[88,89] and the information disseminated by cancer in circulating sources.[90] One advantage of aptamers is the abundance of its targets.[91] Aptamers can bind to advanced glycation end products (AGEs),[81] carcinoembryonic antigen (CEA),[82] vascular endothelial growth factor,[92] myeloid suppressor cells, and tumor-associated macrophages.[93] Once precise diagnosis is accomplished, aptamers may accordingly provide therapy options.

Aptamers can target a wide variety of cells. Zou et al. used electroporation to load chemotherapeutic drugs into exosomes, and then modified aptamers that specifically target tumors with exosomes.[94] The exosomes in this study were derived from immature dendritic cells, and the aptamer was an sgc8 aptamer that specifically recognizes membrane-expressed protein tyrosine kinase 7. The protein tyrosine kinase-7 (PTK7) gene is involved in the occurrence, development, and invasion of many tumors. In addition to cancer cells, other tumor-related cells can be targeted. Recently, Codiak BioSciences, a biotechnology company developing exosomal therapies,

https://dx.doi.org/10.1021/acssensors.0c02237
ACS Sens. 2021, 6, 1418−1429

demonstrated that engineered exosomes loaded with antisense oligonucleotides (ASO) selectively reprogrammed tumor-associated macrophages, which have antitumor activity. Hu et al. observed that exosomes from the tumor necrosis factor-like weak inducer of apoptosis (TWEAK)-stimulated macrophages can be internalized by epithelial ovarian cancer cells and inhibit cell metastasis.[95] Xie et al. reported an innovative method of using aptamer-functionalized nanoparticles to excrete carcinogenic exosomes in the blood into the small intestine.[96] They designed a biomaterial that can accurately identify circulating carcinogenic exosomes in the blood and drag and discard them into the small intestine. We summarize examples of aptamer exosomes used in cancer therapy (Table 1).

In the future, the application of aptamer−exosome therapy may target cancer cells, tumor-associated stromal cells, or tumor-associated immune cells. This therapy may even target the seeds of cancer circulating in the circulatory system, such as CTCs or tumor-derived exosomes. We look forward to the emergence of a personalized aptamer−exosome therapy platform based on the precise diagnosis of cancer in the future (Figure 4).

APTAMER−EXOSOMES: INTEGRATING DIAGNOSTIC AGENTS AND THERAPEUTIC AGENTS FOR FUTURE TUMOR THERANOSTICS

There are many therapeutic approaches for cancer treatment, such as surgery, chemotherapy, radiotherapy, gene therapy, and immunotherapy. However, the efficacy of these treatments is limited. In addition, it is often difficult to determine the outcomes of these treatments in a timely manner. Therefore, in situ and real-time monitoring of targeted therapies has become crucial for treating cancer patients. Compared with a single diagnosis or treatment method, theranostics have obvious advantages. The core technology for theranostics is the ingenious integration of diagnostic and therapeutic agents on the same nanocarrier. Photodynamic therapy and photothermal therapy based on external stimuli have made great progress in tumor nanotherapy.[97,98] For example, Liu et al. used the heavy atom effect of Ta in Ta_4C_3, which could be used for CT imaging, and this nanomaterial has excellent light-to-heat conversion efficiency.[99] In the future, exosomes, contrast agents, and drugs can be used to construct diagnostic and therapeutic integrated nanoparticles. Aptamers are used for targeted delivery, exosomes can protect their contents, and contrast agents are used for tumor imaging. The contrast methods include fluorescence, CT, surface-enhanced Raman scattering (SERS), and drugs that can include chemotherapy, photothermal, or photodynamic materials (Figure 5). We summarize the advantages and disadvantages of aptamer−exosomes for diagnosis and therapy (Table 2).

Table 2. Advantages and Disadvantages of Aptamer−Exosomes in Diagnosis and Therapy

	advantages	disadvantages
Basic characteristic	Structurally stable	Preparation of exosomes takes a long time
	Easy to synthesize	Lack of standardization makes it difficult to commercialize
	Enriched combinatorial forms	The binding site of aptamer and exosome needs to be determined
Diagnosis	High affinity and high specificity	The acquisition of exosomes of disease-specific origin is not addressed
	Ability of renaturation after denaturation	Immature preprocessing step
	Suitable for biosensor design	Expensive instrumentation
Therapy	Nanoscale size	Degraded easily by nucleases
	Low immunogenicity	Short half-life without modification
	Potential acellular vaccines	The specific mechanism of delivery was not studied in depth
	Strong tissue penetration	Delivery mechanisms that are not thoroughly studied

CONCLUSIONS AND FUTURE PERSPECTIVES

Here, we summarize the present studies and future prospects of aptamer−exosomes in cancer diagnosis and treatment. In tumor diagnosis, the current clinical diagnosis approach might only collect exosomes from a subpopulation of cancer tissues and cannot be used to identify tumor-derived exosomes. The combination of aptamers and exosomes could improve the current approach to collecting tumor information. In particular, the aptamer−exosome relying on the microfluidic chip will greatly facilitate the long-term monitoring of tumor patients, particularly for point-of-care testing. By performing multiple tests to produce continuous dynamic data, this technology can achieve dynamic tumor monitoring. In tumor therapy, aptamer−exosomes and targeted cells may be described as key−lock relationships. The goal of this design is for one type of key to only open one specific type of lock. Exosomes can protect the drugs inside such that the drugs are not released easily, reducing the adverse effects on normal cells; aptamers can increase the targeting of drug delivery systems, especially the multiple targeting aptamers in personalized therapy. However, this approach had some limitations. The main problem is that the aptamer selection process is very complicated and only focuses on a specific target. Although the targets of aptamers produced by cell-SELEX are quite abundant, there are still many problems to be solved when the binding complex of aptamers and exosomes needs to be deconstructed. In the text, we discuss how to identify the targets of aptamers generated by cell-SELEX, including SDS-PAGE, protein sequencing, and validation. However, this presupposes that the target of the aptamer is a protein and ignores other possibilities. To investigate the interactions between aptamers and targets, a range of physicochemical methods has been routinely applied. Currently, NMR spectroscopy and X-ray crystallography are two of the most important structural analysis methods.[100] However, neither X-ray crystal diffraction nor NMR is well suited for studying nucleic acid structures. Only small fragments of nucleic acid sequences can be studied using the NM. If X-ray is used, nucleic acid aptamers are required to be able to form crystals, but most nucleic acid aptamers are unable to form crystals. The Cryo EM technique is a very pyrogenic means of studying macromolecular structures, while it is difficult to study aptamer by Cryo EM due to the low limit of detection. Although not reaching atomic-level resolution, researchers have made it possible to analyze the overall three-dimensional structure of RNA molecules using Cryo EM, as well as the conformational changes induced by the binding of RNA to other factors.[101] We look forward to breakthroughs in nucleic acid structural research techniques to provide more detailed information on aptamer target interactions. Another

https://dx.doi.org/10.1021/acssensors.0c02237
ACS Sens. 2021, 6, 1418−1429

issue is that the specific mechanism underlying cell-mediated uptake of exosomes has not yet been elucidated. For exosome-mediated drug delivery, it is critical to determine the mechanism by which exosomes are preferentially taken up by specific target organs. Whether this targetability is altered after modification, especially after aptamer modification, also needs to be explored. Although this phenomenon can be determined by observation of characteristics such as fluorescence for aptamer−exosome, the specific mechanism requires further research.

The most promising application of aptamer−exosomes is for the integration of diagnosis and treatment. Patient-centered diagnosis and treatment have been a trend in the field of oncology. To achieve this goal, accurate diagnosis and personalized treatment are required. In the future, microfluidic chips based on aptamer panels can be developed to screen responding aptamers, and personalized targeted theranostic nanoparticles can be formulated using these aptamers.

In general, the prospect of aptamer−exosomes is promising, and it may expand to more applications for tumor diagnosis and treatment.

AUTHOR INFORMATION

Corresponding Authors

Fubing Wang − *Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan 430071, P.R. China; Wuhan Research Center for Infectious Diseases and Cancer, Chinese Academy of Medical Sciences, Wuhan 430071, P.R. China;* orcid.org/0000-0002-5971-2622; Phone: +86-27-67813517; Email: wfb20042002@sina.com

Jianyuan Wu − *Clinical Trial Center of Zhongnan Hospital, Wuhan University, Wuhan 430071, P.R. China*; Email: wujianyuan@znhospital.com

Authors

Kezhen Yi − *Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan 430071, P.R. China*

Yuan Rong − *Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan 430071, P.R. China*

Lanxiang Huang − *Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan 430071, P.R. China*

Xuan Tang − *Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan 430071, P.R. China*

Qian Zhang − *Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan 430071, P.R. China*

Wei Wang − *Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan 430071, P.R. China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acssensors.0c02237

Author Contributions

#K.Y. and Y.R. contributed equally. F.W. and J.W. contributed to the conception of the study. L.H., X.T., Q.Z., and W.W. contributed significantly to analysis and manuscript preparation. K.Y. performed the data analyses and wrote the manuscript. All authors have given approval to the final version of the manuscript.

Funding

This work was supported by the Improvement Project for Theranostic ability on Difficulty Miscellaneous disease (Tumor) and the Science & Technology Innovation Fostering Foundation of Zhongnan Hospital of Wuhan University (ZLYNXM202008, No. znpy2019050, znpy2019004, znpy2018027), This work was also funded by Medical Top-talented youth development project of Hubei Province, and by Medical talented youth development project in Health Commission of Hubei Province (No. WJ2019Q049).

Notes

The authors declare no competing financial interest.

ABBREVIATIONS

AFP, alpha-fetoprotein; AGEs, advanced glycation end products; ASO, antisense oligonucleotides; CAR-T, chimeric antigen reeptor T-cell immunotherapy; CD47, cluster of differentiation 47; CD63, cluster of differentiation 63; CEA, carcinoembryonic antigen; CRISPR, clustered regularly interspaced short palindromic repeats; CT, computed tomography; CTC, circulating tumor cell; EpCAM, epithelial cell adhesion molecule; GPC1, glypican 1; HCC, hepatocellular carcinoma; KRAS, kirsten rat sarcoma viral oncogene; MSCs, mesenchymal stem cells; MUC1, mucin-1; NK cell, natural killer cell; PCAT-1, prostate cancer-associated transcript 1; PD-1, programmed death receptor 1; PD-L1, programmed cell death 1 ligand 1; PSMA, prostate specific membrane antigen; SELEX, systematic evolution of ligands by exponential enrichment; SERS, surface-enhanced Raman scattering; SPR, surface plasmon resonance; TWEAK, tumor necrosis factor-like weak inducer of apoptosis; MALAT1, metastasis associated lung adenocarcinoma transcript 1; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

VOCABULARY

Aptamer, Aptamers are a short stretch of oligonucleotide sequences or short polypeptides that have been selected in vitro and can bind with high affinity and strong specificity to their corresponding ligands.; Exosome, Exosomes are nano-sized vesicular structures secreted by cells and are found in various body fluids such as blood, saliva, and urine. As one of the important intercellular communication tools, exosomes contain various bioactive components such as nucleic acids and proteins, which can play a regulatory role in the human body through many ways.; Biosensor, Biosensor is an instrument that is sensitive to biological substances and converts their concentration or other information into electrical signals for detection. Biosensors are analytical tools or systems composed of immobilized biosensitive materials as recognition elements, appropriate physicochemical transducers, and signal amplification devices.; Microfluidic chip, Microfluidic chip technology is the integration of the basic operational units, such as sample preparation, reaction, separation, and detection, of biological, chemical, and medical analytical processes into a single micrometer scale chip, automatically completing the whole process of analysis.; SELEX, SELEX technology is the technique of systematic evolution of ligands by exponential enrichment. With this technique, aptamers can be screened from a library of random single stranded nucleic acid sequences with high affinity to the target specifically.

REFERENCES

(1) Shao, H.; Im, H.; Castro, C. M.; Breakefield, X.; Weissleder, R.; Lee, H. New Technologies for Analysis of Extracellular Vesicles. *Chem. Rev.* **2018**, *118* (4), 1917−1950.

(2) Théry, C.; Witwer, K. W.; Aikawa, E.; Alcaraz, M. J.; Anderson, J. D.; Andriantsitohaina, R.; Antoniou, A.; Arab, T.; Archer, F.; Atkin-

Smith, G. K.; Ayre, D. C.; Bach, J. M.; Bachurski, D.; Baharvand, H.; Balaj, L.; Baldacchino, S.; Bauer, N. N.; Baxter, A. A.; Bebawy, M.; Beckham, C.; Bedina Zavec, A.; Benmoussa, A.; Berardi, A. C.; Bergese, P.; Bielska, E.; Blenkiron, C.; Bobis-Wozowicz, S.; Boilard, E.; Boireau, W.; Bongiovanni, A.; Borràs, F. E.; Bosch, S.; Boulanger, C. M.; Breakefield, X.; Breglio, A. M.; Brennan, M.; Brigstock, D. R.; Brisson, A.; Broekman, M. L.; Bromberg, J. F.; Bryl-Górecka, P.; Buch, S.; Buck, A. H.; Burger, D.; Busatto, S.; Buschmann, D.; Bussolati, B.; Buzás, E. I.; Byrd, J. B.; Camussi, G.; Carter, D. R.; Caruso, S.; Chamley, L. W.; Chang, Y. T.; Chen, C.; Chen, S.; Cheng, L.; Chin, A. R.; Clayton, A.; Clerici, S. P.; Cocks, A.; Cocucci, E.; Coffey, R. J.; Cordeiro-Da-Silva, A.; Couch, Y.; Coumans, F. A.; Coyle, B.; Crescitelli, R.; Criado, M. F.; D'Souza-Schorey, C.; Das, S.; Datta Chaudhuri, A.; de Candia, P.; De Santana, E. F.; De Wever, O.; Del Portillo, H. A.; Demaret, T.; Deville, S.; Devitt, A.; Dhondt, B.; Di Vizio, D.; Dieterich, L. C.; Dolo, V.; Dominguez Rubio, A. P.; Dominici, M.; Dourado, M. R.; Driedonks, T. A.; Duarte, F. V.; Duncan, H. M.; Eichenberger, R. M.; Ekström, K.; El Andaloussi, S.; Elie-Caille, C.; Erdbrügger, U.; Falcón-Pérez, J. M.; Fatima, F.; Fish, J. E.; Flores-Bellver, M.; Försönits, A.; Frelet-Barrand, A.; Fricke, F.; Fuhrmann, G.; Gabrielsson, S.; Gámez-Valero, A.; Gardiner, C.; Gärtner, K.; Gaudin, R.; Gho, Y. S.; Giebel, B.; Gilbert, C.; Gimona, M.; Giusti, I.; Goberdhan, D. C.; Görgens, A.; Gorski, S. M.; Greening, D. W.; Gross, J. C.; Gualerzi, A.; Gupta, G. N.; Gustafson, D.; Handberg, A.; Haraszti, R. A.; Harrison, P.; Hegyesi, H.; Hendrix, A.; Hill, A. F.; Hochberg, F. H.; Hoffmann, K. F.; Holder, B.; Holthofer, H.; Hosseinkhani, B.; Hu, G.; Huang, Y.; Huber, V.; Hunt, S.; Ibrahim, A. G.; Ikezu, T.; Inal, J. M.; Isin, M.; Ivanova, A.; Jackson, H. K.; Jacobsen, S.; Jay, S. M.; Jayachandran, M.; Jenster, G.; Jiang, L.; Johnson, S. M.; Jones, J. C.; Jong, A.; Jovanovic-Talisman, T.; Jung, S.; Kalluri, R.; Kano, S. I.; Kaur, S.; Kawamura, Y.; Keller, E. T.; Khamari, D.; Khomyakova, E.; Khvorova, A.; Kierulf, P.; Kim, K. P.; Kislinger, T.; Klingeborn, M.; Klinke, D. J., 2nd; Kornek, M.; Kosanović, M. M.; Kovács Á, F.; Krämer-Albers, E. M.; Krasemann, S.; Krause, M.; Kurochkin, I. V.; Kusuma, G. D.; Kuypers, S.; Laitinen, S.; Langevin, S. M.; Languino, L. R.; Lannigan, J.; Lässer, C.; Laurent, L. C.; Lavieu, G.; Lázaro-Ibáñez, E.; Le Lay, S.; Lee, M. S.; Lee, Y. X. F.; Lemos, D. S.; Lenassi, M.; Leszczynska, A.; Li, I. T.; Liao, K.; Libregts, S. F.; Ligeti, E.; Lim, R.; Lim, S. K.; Linē, A.; Linnemannstöns, K.; Llorente, A.; Lombard, C. A.; Lorenowicz, M. J.; Lörincz Á, M.; Lötvall, J.; Lovett, J.; Lowry, M. C.; Loyer, X.; Lu, Q.; Lukomska, B.; Lunavat, T. R.; Maas, S. L.; Malhi, H.; Marcilla, A.; Mariani, J.; Mariscal, J.; Martens-Uzunova, E. S.; Martin-Jaular, L.; Martinez, M. C.; Martins, V. R.; Mathieu, M.; Mathivanan, S.; Maugeri, M.; McGinnis, L. K.; McVey, M. J.; Meckes, D. G., Jr.; Meehan, K. L.; Mertens, I.; Minciacchi, V. R.; Möller, A.; Møller Jørgensen, M.; Morales-Kastresana, A.; Morhayim, J.; Mullier, F.; Muraca, M.; Musante, L.; Mussack, V.; Muth, D. C.; Myburgh, K. H.; Najrana, T.; Nawaz, M.; Nazarenko, I.; Nejsum, P.; Neri, C.; Neri, T.; Nieuwland, R.; Nimrichter, L.; Nolan, J. P.; Nolte-'t Hoen, E. N.; Noren Hooten, N.; O'Driscoll, L.; O'Grady, T.; O'Loghlen, A.; Ochiya, T.; Olivier, M.; Ortiz, A.; Ortiz, L. A.; Osteikoetxea, X.; Østergaard, O.; Ostrowski, M.; Park, J.; Pegtel, D. M.; Peinado, H.; Perut, F.; Pfaffl, M. W.; Phinney, D. G.; Pieters, B. C.; Pink, R. C.; Pisetsky, D. S.; Pogge von Strandmann, E.; Polakovicova, I.; Poon, I. K.; Powell, B. H.; Prada, I.; Pulliam, L.; Quesenberry, P.; Radeghieri, A.; Raffai, R. L.; Raimondo, S.; Rak, J.; Ramirez, M. I.; Raposo, G.; Rayyan, M. S.; Regev-Rudzki, N.; Ricklefs, F. L.; Robbins, P. D.; Roberts, D. D.; Rodrigues, S. C.; Rohde, E.; Rome, S.; Rouschop, K. M.; Rughetti, A.; Russell, A. E.; Saá, P.; Sahoo, S.; Salas-Huenuleo, E.; Sánchez, C.; Saugstad, J. A.; Saul, M. J.; Schiffelers, R. M.; Schneider, R.; Schøyen, T. H.; Scott, A.; Shahaj, E.; Sharma, S.; Shatnyeva, O.; Shekari, F.; Shelke, G. V.; Shetty, A. K.; Shiba, K.; Siljander, P. R.; Silva, A. M.; Skowronek, A.; Snyder, O. L., 2nd; Soares, R. P.; Sódar, B. W.; Soekmadji, C.; Sotillo, J.; Stahl, P. D.; Stoorvogel, W.; Stott, S. L.; Strasser, E. F.; Swift, S.; Tahara, H.; Tewari, M.; Timms, K.; Tiwari, S.; Tixeira, R.; Tkach, M.; Toh, W. S.; Tomasini, R.; Torrecilhas, A. C.; Tosar, J. P.; Toxavidis, V.; Urbanelli, L.; Vader, P.; van Balkom, B. W.; van der Grein, S. G.; Van Deun, J.; van Herwijnen, M. J.; Van Keuren-Jensen, K.; van Niel, G.; van Royen, M. E.; van Wijnen, A. J.; Vasconcelos, M. H.; Vechetti, I. J., Jr.; Veit, T. D.; Vella, L. J.; Velot, É.; Verweij, F. J.; Vestad, B.; Viñas, J. L.; Visnovitz, T.; Vukman, K. V.; Wahlgren, J.; Watson, D. C.; Wauben, M. H.; Weaver, A.; Webber, J. P.; Weber, V.; Wehman, A. M.; Weiss, D. J.; Welsh, J. A.; Wendt, S.; Wheelock, A. M.; Wiener, Z.; Witte, L.; Wolfram, J.; Xagorari, A.; Xander, P.; Xu, J.; Yan, X.; Yáñez-Mó, M.; Yin, H.; Yuana, Y.; Zappulli, V.; Zarubova, J.; Žėkas, V.; Zhang, J. Y.; Zhao, Z.; Zheng, L.; Zheutlin, A. R.; Zickler, A. M.; Zimmermann, P.; Zivkovic, A. M.; Zocco, D.; Zuba-Surma, E. K. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J. Extracell. Vesicles* **2018**, *7* (1), 1535750.

(3) Tkach, M.; Théry, C. Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. *Cell* **2016**, *164* (6), 1226−1232.

(4) Ruivo, C. F.; Adem, B.; Silva, M.; Melo, S. A. The Biology of Cancer Exosomes: Insights and New Perspectives. *Cancer Res.* **2017**, *77* (23), 6480−6488.

(5) Becker, A.; Thakur, B. K.; Weiss, J. M.; Kim, H. S.; Peinado, H.; Lyden, D. Extracellular Vesicles in Cancer: Cell-to-Cell Mediators of Metastasis. *Cancer Cell* **2016**, *30* (6), 836−848.

(6) Sheridan, C. Exosome cancer diagnostic reaches market. *Nat. Biotechnol.* **2016**, *34* (4), 359−60.

(7) Tran, P. H. L.; Xiang, D.; Tran, T. T. D.; Yin, W.; Zhang, Y.; Kong, L.; Chen, K.; Sun, M.; Li, Y.; Hou, Y.; Zhu, Y.; Duan, W. Exosomes and Nanoengineering: A Match Made for Precision Therapeutics. *Adv. Mater. (Weinheim, Ger.)* **2020**, *32*, 1904040.

(8) Vagner, T.; Spinelli, C.; Minciacchi, V. R.; Balaj, L.; Zandian, M.; Conley, A.; Zijlstra, A.; Freeman, M. R.; Demichelis, F.; De, S.; Posadas, E. M.; Tanaka, H.; Di Vizio, D. Large extracellular vesicles carry most of the tumour DNA circulating in prostate cancer patient plasma. *J. Extracell. Vesicles* **2018**, *7* (1), 1505403.

(9) McKiernan, J.; Donovan, M. J.; O'Neill, V.; Bentink, S.; Noerholm, M.; Belzer, S.; Skog, J.; Kattan, M. W.; Partin, A.; Andriole, G.; Brown, G.; Wei, J. T.; Thompson, I. M.; Carroll, P. A Novel Urine Exosome Gene Expression Assay to Predict High-grade Prostate Cancer at Initial Biopsy. *JAMA oncology* **2016**, *2* (7), 882−9.

(10) Sun, Y.; Liu, S.; Qiao, Z.; Shang, Z.; Xia, Z.; Niu, X.; Qian, L.; Zhang, Y.; Fan, L.; Cao, C. X.; Xiao, H. Systematic comparison of exosomal proteomes from human saliva and serum for the detection of lung cancer. *Anal. Chim. Acta* **2017**, *982*, 84−95.

(11) Liu, C.; Xu, X.; Li, B.; Situ, B.; Pan, W.; Hu, Y.; An, T.; Yao, S.; Zheng, L. Single-Exosome-Counting Immunoassays for Cancer Diagnostics. *Nano Lett.* **2018**, *18* (7), 4226−4232.

(12) Zhu, L.; Li, J.; Gong, Y.; Wu, Q.; Tan, S.; Sun, D.; Xu, X.; Zuo, Y.; Zhao, Y.; Wei, Y. Q.; Wei, X. W.; Peng, Y. Exosomal tRNA-derived small RNA as a promising biomarker for cancer diagnosis. *Mol. Cancer* **2019**, *18* (1), 74.

(13) Zheng, M.; Hou, L.; Ma, Y.; Zhou, L.; Wang, F.; Cheng, B.; Wang, W.; Lu, B.; Liu, P.; Lu, W.; Lu, Y. Exosomal let-7d-3p and miR-30d-5p as diagnostic biomarkers for non-invasive screening of cervical cancer and its precursors. *Mol. Cancer* **2019**, *18* (1), 76.

(14) Wang, L.; Li, Y.; Guan, X.; Zhao, J.; Shen, L.; Liu, J. Exosomal double-stranded DNA as a biomarker for the diagnosis and preoperative assessment of pheochromocytoma and paraganglioma. *Mol. Cancer* **2018**, *17* (1), 128.

(15) Chen, G.; Huang, A. C.; Zhang, W.; Zhang, G.; Wu, M.; Xu, W.; Yu, Z.; Yang, J.; Wang, B.; Sun, H.; Xia, H.; Man, Q.; Zhong, W.; Antelo, L. F.; Wu, B.; Xiong, X.; Liu, X.; Guan, L.; Li, T.; Liu, S.; Yang, R.; Lu, Y.; Dong, L.; McGettigan, S.; Somasundaram, R.; Radhakrishnan, R.; Mills, G.; Lu, Y.; Kim, J.; Chen, Y. H.; Dong, H.; Zhao, Y.; Karakousis, G. C.; Mitchell, T. C.; Schuchter, L. M.; Herlyn, M.; Wherry, E. J.; Xu, X.; Guo, W. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. *Nature* **2018**, *560* (7718), 382−386.

(16) He, C.; Zheng, S.; Luo, Y.; Wang, B. Exosome Theranostics: Biology and Translational Medicine. *Theranostics* **2018**, *8* (1), 237−255.

https://dx.doi.org/10.1021/acssensors.0c02237
ACS Sens. 2021, 6, 1418−1429

(17) Crivianu-Gaita, V.; Thompson, M. Aptamers, antibody scFv, and antibody Fab' fragments: An overview and comparison of three of the most versatile biosensor biorecognition elements. *Biosens. Bioelectron.* **2016**, *85*, 32−45.
(18) Bunka, D. H.; Stockley, P. G. Aptamers come of age - at last. *Nat. Rev. Microbiol.* **2006**, *4* (8), 588−96.
(19) Huang, R.; He, L.; Xia, Y.; Xu, H.; Liu, C.; Xie, H.; Wang, S.; Peng, L.; Liu, Y.; Liu, Y.; He, N.; Li, Z. A Sensitive Aptasensor Based on a Hemin/G-Quadruplex-Assisted Signal Amplification Strategy for Electrochemical Detection of Gastric Cancer Exosomes. *Small (Weinheim an der Bergstrasse, Germany)* **2019**, *15* (19), No. e1900735.
(20) Li, P.; Yu, X.; Han, W.; Kong, Y.; Bao, W.; Zhang, J.; Zhang, W.; Gu, Y. Ultrasensitive and Reversible Nanoplatform of Urinary Exosomes for Prostate Cancer Diagnosis. *ACS sensors* **2019**, *4* (5), 1433−1441.
(21) White, R.; Rusconi, C.; Scardino, E.; Wolberg, A.; Lawson, J.; Hoffman, M.; Sullenger, B. Generation of species cross-reactive aptamers using "toggle" SELEX. *Mol. Ther.* **2001**, *4* (6), 567−73.
(22) Farokhzad, O. C.; Jon, S.; Khademhosseini, A.; Tran, T. N.; Lavan, D. A.; Langer, R. Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells. *Cancer Res.* **2004**, *64* (21), 7668−72.
(23) Zou, J.; Shi, M.; Liu, X.; Jin, C.; Xing, X.; Qiu, L.; Tan, W. Aptamer-Functionalized Exosomes: Elucidating the Cellular Uptake Mechanism and the Potential for Cancer-Targeted Chemotherapy. *Anal. Chem.* **2019**, *91* (3), 2425−2430.
(24) Feng, W.; Dean, D. C.; Hornicek, F. J.; Shi, H.; Duan, Z. Exosomes promote pre-metastatic niche formation in ovarian cancer. *Mol. Cancer* **2019**, *18* (1), 124.
(25) Morad, G.; Moses, M. A. Brainwashed by extracellular vesicles: the role of extracellular vesicles in primary and metastatic brain tumour microenvironment. *J. Extracell. Vesicles* **2019**, *8* (1), 1627164.
(26) Han, L.; Lam, E. W.; Sun, Y. Extracellular vesicles in the tumor microenvironment: old stories, but new tales. *Mol. Cancer* **2019**, *18* (1), 59.
(27) Zhang, J.; Li, S.; Li, L.; Li, M.; Guo, C.; Yao, J.; Mi, S. Exosome and exosomal microRNA: trafficking, sorting, and function. *Genomics, Proteomics Bioinf.* **2015**, *13* (1), 17−24.
(28) Melo, S. A.; Luecke, L. B.; Kahlert, C.; Fernandez, A. F.; Gammon, S. T.; Kaye, J.; LeBleu, V. S.; Mittendorf, E. A.; Weitz, J.; Rahbari, N.; Reissfelder, C.; Pilarsky, C.; Fraga, M. F.; Piwnica-Worms, D.; Kalluri, R. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. *Nature* **2015**, *523* (7559), 177−182.
(29) Melo, S. A.; Luecke, L. B.; Kahlert, C.; Fernandez, A. F.; Gammon, S. T.; Kaye, J.; LeBleu, V. S.; Mittendorf, E. A.; Weitz, J.; Rahbari, N.; Reissfelder, C.; Pilarsky, C.; Fraga, M. F.; Piwnica-Worms, D.; Kalluri, R. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. *Nature* **2015**, *523* (7559), 177−82.
(30) Jakobsen, K. R.; Paulsen, B. S.; Baek, R.; Varming, K.; Sorensen, B. S.; Jorgensen, M. M. Exosomal proteins as potential diagnostic markers in advanced non-small cell lung carcinoma. *J. Extracell. Vesicles* **2015**, *4*, 26659.
(31) Taylor, D. D.; Gercel-Taylor, C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecol. Oncol.* **2008**, *110* (1), 13−21.
(32) Skotland, T.; Ekroos, K.; Kauhanen, D.; Simolin, H.; Seierstad, T.; Berge, V.; Sandvig, K.; Llorente, A. Molecular lipid species in urinary exosomes as potential prostate cancer biomarkers. *Eur. J. Cancer* **2017**, *70*, 122−132.
(33) Zhan, Y.; Du, L.; Wang, L.; Jiang, X.; Zhang, S.; Li, J.; Yan, K.; Duan, W.; Zhao, Y.; Wang, L.; Wang, Y.; Wang, C. Expression signatures of exosomal long non-coding RNAs in urine serve as novel non-invasive biomarkers for diagnosis and recurrence prediction of bladder cancer. *Mol. Cancer* **2018**, *17* (1), 142.
(34) Lee, K.; Fraser, K.; Ghaddar, B.; Yang, K.; Kim, E.; Balaj, L.; Chiocca, E. A.; Breakefield, X. O.; Lee, H.; Weissleder, R. Multiplexed Profiling of Single Extracellular Vesicles. *ACS Nano* **2018**, *12* (1), 494−503.
(35) Wang, Z.; Sun, X.; Natalia, A.; Tang, C. S. L.; Ang, C. B. T.; Ong, C.-A. J.; Teo, M. C. C.; So, J. B. Y.; Shao, H. Dual-Selective Magnetic Analysis of Extracellular Vesicle Glycans. *Matter* **2020**, *2* (1), 150−166.
(36) Wu, X.; Zhao, H.; Natalia, A.; Lim, C. Z. J.; Ho, N. R. Y.; Ong, C. J.; Teo, M. C. C.; So, J. B. Y.; Shao, H. Exosome-templated nanoplasmonics for multiparametric molecular profiling. *Science advances* **2020**, *6* (19), No. eaba2556.
(37) Kim, B.; Jung, I. H.; Kang, M.; Shim, H. K.; Woo, H. Y. Cationic conjugated polyelectrolytes-triggered conformational change of molecular beacon aptamer for highly sensitive and selective potassium ion detection. *J. Am. Chem. Soc.* **2012**, *134* (6), 3133−8.
(38) Dong, L.; Tan, Q.; Ye, W.; Liu, D.; Chen, H.; Hu, H.; Wen, D.; Liu, Y.; Cao, Y.; Kang, J.; Fan, J.; Guo, W.; Wu, W. Screening and Identifying a Novel ssDNA Aptamer against Alpha-fetoprotein Using CE-SELEX. *Sci. Rep.* **2015**, *5*, 15552.
(39) Li, W. M.; Bing, T.; Wei, J. Y.; Chen, Z. Z.; Shangguan, D. H.; Fang, J. Cell-SELEX-based selection of aptamers that recognize distinct targets on metastatic colorectal cancer cells. *Biomaterials* **2014**, *35* (25), 6998−7007.
(40) Liang, C.; Guo, B.; Wu, H.; Shao, N.; Li, D.; Liu, J.; Dang, L.; Wang, C.; Li, H.; Li, S.; Lau, W. K.; Cao, Y.; Yang, Z.; Lu, C.; He, X.; Au, D. W.; Pan, X.; Zhang, B. T.; Lu, C.; Zhang, H.; Yue, K.; Qian, A.; Shang, P.; Xu, J.; Xiao, L.; Bian, Z.; Tan, W.; Liang, Z.; He, F.; Zhang, L.; Lu, A.; Zhang, G. Aptamer-functionalized lipid nanoparticles targeting osteoblasts as a novel RNA interference-based bone anabolic strategy. *Nat. Med.* **2015**, *21* (3), 288−94.
(41) Wang, F. B.; Rong, Y.; Fang, M.; Yuan, J. P.; Peng, C. W.; Liu, S. P.; Li, Y. Recognition and capture of metastatic hepatocellular carcinoma cells using aptamer-conjugated quantum dots and magnetic particles. *Biomaterials* **2013**, *34* (15), 3816−27.
(42) Lyu, Y.; Chen, G.; Shangguan, D.; Zhang, L.; Wan, S.; Wu, Y.; Zhang, H.; Duan, L.; Liu, C.; You, M.; Wang, J.; Tan, W. Generating Cell Targeting Aptamers for Nanotheranostics Using Cell-SELEX. *Theranostics* **2016**, *6* (9), 1440−52.
(43) Tan, W.; Donovan, M. J.; Jiang, J. Aptamers from cell-based selection for bioanalytical applications. *Chem. Rev.* **2013**, *113* (4), 2842−62.
(44) Dong, H.; Chen, H.; Jiang, J.; Zhang, H.; Cai, C.; Shen, Q. Highly Sensitive Electrochemical Detection of Tumor Exosomes Based on Aptamer Recognition-Induced Multi-DNA Release and Cyclic Enzymatic Amplification. *Anal. Chem.* **2018**, *90* (7), 4507−4513.
(45) Wang, F.-B.; Rong, Y.; Fang, M.; Yuan, J.-P.; Peng, C.-W.; Liu, S.-P.; Li, Y. Recognition and capture of metastatic hepatocellular carcinoma cells using aptamer-conjugated quantum dots and magnetic particles. *Biomaterials* **2013**, *34* (15), 3816−3827.
(46) Zhang, K.; Yue, Y.; Wu, S.; Liu, W.; Shi, J.; Zhang, Z. Rapid Capture and Nondestructive Release of Extracellular Vesicles Using Aptamer-Based Magnetic Isolation. *ACS sensors* **2019**, *4* (5), 1245−1251.
(47) Darmostuk, M.; Rimpelova, S.; Gbelcova, H.; Ruml, T. Current approaches in SELEX: An update to aptamer selection technology. *Biotechnol. Adv.* **2015**, *33*, 1141−61.
(48) Chen, M.; Yu, Y.; Jiang, F.; Zhou, J.; Li, Y.; Liang, C.; Dang, L.; Lu, A.; Zhang, G. Development of Cell-SELEX Technology and Its Application in Cancer Diagnosis and Therapy. *Int. J. Mol. Sci.* **2016**, *17* (12), 2079.
(49) Sun, H.; Zhu, X.; Lu, P. Y.; Rosato, R. R.; Tan, W.; Zu, Y. Oligonucleotide aptamers: new tools for targeted cancer therapy. *Mol. Ther.–Nucleic Acids* **2014**, *3* (8), No. e182.
(50) Fang, X.; Tan, W. Aptamers generated from cell-SELEX for molecular medicine: a chemical biology approach. *Acc. Chem. Res.* **2010**, *43* (1), 48−57.
(51) Wang, H.; Chen, H.; Huang, Z.; Li, T.; Deng, A.; Kong, J. DNase I enzyme-aided fluorescence signal amplification based on graphene oxide-DNA aptamer interactions for colorectal cancer exosome detection. *Talanta* **2018**, *184*, 219−226.

https://dx.doi.org/10.1021/acssensors.0c02237
ACS Sens. 2021, 6, 1418−1429

(52) Liao, G.; Liu, X.; Yang, X.; Wang, Q.; Geng, X.; Zou, L.; Liu, Y.; Li, S.; Zheng, Y.; Wang, K. Surface plasmon resonance assay for exosomes based on aptamer recognition and polydopamine-functionalized gold nanoparticles for signal amplification. *Microchim. Acta* **2020**, *187* (4), 251.
(53) Zhu, K.; Wang, Z.; Zong, S.; Liu, Y.; Yang, K.; Li, N.; Wang, Z.; Li, L.; Tang, H.; Cui, Y. Hydrophobic Plasmonic Nanoacorn Array for a Label-Free and Uniform SERS-Based Biomolecular Assay. *ACS Appl. Mater. Interfaces* **2020**, *12* (26), 29917−29927.
(54) Chen, W.; Li, J.; Wei, X.; Fan, Y.; Qian, H.; Li, S.; Xiang, Y.; Ding, S. Surface plasmon resonance biosensor using hydrogel-AuNP supramolecular spheres for determination of prostate cancer-derived exosomes. *Microchim. Acta* **2020**, *187* (11), 590.
(55) Chen, J.; Meng, H. M.; An, Y.; Geng, X.; Zhao, K.; Qu, L.; Li, Z. Structure-switching aptamer triggering hybridization displacement reaction for label-free detection of exosomes. *Talanta* **2020**, *209*, 120510.
(56) Zhang, H.; Wang, Z.; Wang, F.; Zhang, Y.; Wang, H.; Liu, Y. In Situ Formation of Gold Nanoparticles Decorated Ti(3)C(2) MXenes Nanoprobe for Highly Sensitive Electrogenerated Chemiluminescence Detection of Exosomes and Their Surface Proteins. *Anal. Chem.* **2020**, *92* (7), 5546−5553.
(57) Huang, R.; He, L.; Li, S.; Liu, H.; Jin, L.; Chen, Z.; Zhao, Y.; Li, Z.; Deng, Y.; He, N. A simple fluorescence aptasensor for gastric cancer exosome detection based on branched rolling circle amplification. *Nanoscale* **2020**, *12* (4), 2445−2451.
(58) Li, B.; Pan, W.; Liu, C.; Guo, J.; Shen, J.; Feng, J.; Luo, T.; Situ, B.; Zhang, Y.; An, T.; Xu, C.; Zheng, W.; Zheng, L. Homogenous Magneto-Fluorescent Nanosensor for Tumor-Derived Exosome Isolation and Analysis. *ACS sensors* **2020**, *5* (7), 2052−2060.
(59) Li, B.; Liu, C.; Pan, W.; Shen, J.; Guo, J.; Luo, T.; Feng, J.; Situ, B.; An, T.; Zhang, Y.; Zheng, L. Facile fluorescent aptasensor using aggregation-induced emission luminogens for exosomal proteins profiling towards liquid biopsy. *Biosens. Bioelectron.* **2020**, *168*, 112520.
(60) Yu, Y.; Zhang, W. S.; Guo, Y.; Peng, H.; Zhu, M.; Miao, D.; Su, G. Engineering of exosome-triggered enzyme-powered DNA motors for highly sensitive fluorescence detection of tumor-derived exosomes. *Biosens. Bioelectron.* **2020**, *167*, 112482.
(61) Jiang, J.; Yu, Y.; Zhang, H.; Cai, C. Electrochemical aptasensor for exosomal proteins profiling based on DNA nanotetrahedron coupled with enzymatic signal amplification. *Anal. Chim. Acta* **2020**, *1130*, 1−9.
(62) Xu, L.; Chopdat, R.; Li, D.; Al-Jamal, K. T. Development of a simple, sensitive and selective colorimetric aptasensor for the detection of cancer-derived exosomes. *Biosens. Bioelectron.* **2020**, *169*, 112576.
(63) Xing, S.; Lu, Z.; Huang, Q.; Li, H.; Wang, Y.; Lai, Y.; He, Y.; Deng, M.; Liu, W. An ultrasensitive hybridization chain reaction-amplified CRISPR-Cas12a aptasensor for extracellular vesicle surface protein quantification. *Theranostics* **2020**, *10* (22), 10262−10273.
(64) Wang, L.; Pan, Y.; Liu, Y.; Sun, Z.; Huang, Y.; Li, J.; Yang, J.; Xiang, Y.; Li, G. Fabrication of an Aptamer-Coated Liposome Complex for the Detection and Profiling of Exosomes Based on Terminal Deoxynucleotidyl Transferase-Mediated Signal Amplification. *ACS Appl. Mater. Interfaces* **2020**, *12* (1), 322−329.
(65) Liu, C.; Zhao, J.; Tian, F.; Cai, L.; Zhang, W.; Feng, Q.; Chang, J.; Wan, F.; Yang, Y.; Dai, B.; Cong, Y.; Ding, B.; Sun, J.; Tan, W. Low-cost thermophoretic profiling of extracellular-vesicle surface proteins for the early detection and classification of cancers. *Nat. Biomed Eng.* **2019**, *3* (3), 183−193.
(66) Rani, S.; Ritter, T. The Exosome - A Naturally Secreted Nanoparticle and its Application to Wound Healing. *Adv. Mater. (Weinheim, Ger.)* **2016**, *28* (27), 5542−52.
(67) Robbins, P. D.; Morelli, A. E. Regulation of immune responses by extracellular vesicles. *Nat. Rev. Immunol.* **2014**, *14* (3), 195−208.
(68) EL Andaloussi, S.; Mager, I.; Breakefield, X. O.; Wood, M. J. A. Extracellular vesicles: biology and emerging therapeutic opportunities. *Nat. Rev. Drug Discovery* **2013**, *12* (5), 347−57.
(69) Thery, C.; Zitvogel, L.; Amigorena, S. Exosomes: composition, biogenesis and function. *Nat. Rev. Immunol.* **2002**, *2* (8), 569−79.
(70) Zitvogel, L.; Regnault, A.; Lozier, A.; Wolfers, J.; Flament, C.; Tenza, D.; Ricciardi-Castagnoli, P.; Raposo, G.; Amigorena, S. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nat. Med.* **1998**, *4* (5), 594−600.
(71) Kamerkar, S.; LeBleu, V. S.; Sugimoto, H.; Yang, S.; Ruivo, C. F.; Melo, S. A.; Lee, J. J.; Kalluri, R. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature* **2017**, *546* (7659), 498−503.
(72) Wang, G.; Hu, W.; Chen, H.; Shou, X.; Ye, T.; Xu, Y. Cocktail Strategy Based on NK Cell-Derived Exosomes and Their Biomimetic Nanoparticles for Dual Tumor Therapy. *Cancers* **2019**, *11* (10), 1560.
(73) Fu, W.; Lei, C.; Liu, S.; Cui, Y.; Wang, C.; Qian, K.; Li, T.; Shen, Y.; Fan, X.; Lin, F.; Ding, M.; Pan, M.; Ye, X.; Yang, Y.; Hu, S. CAR exosomes derived from effector CAR-T cells have potent antitumour effects and low toxicity. *Nat. Commun.* **2019**, *10* (1), 4355.
(74) Kibria, G.; Ramos, E. K.; Wan, Y.; Gius, D. R.; Liu, H. Exosomes as a Drug Delivery System in Cancer Therapy: Potential and Challenges. *Mol. Pharmaceutics* **2018**, *15* (9), 3625−3633.
(75) Alvarez-Erviti, L.; Seow, Y.; Yin, H.; Betts, C.; Lakhal, S.; Wood, M. J. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat. Biotechnol.* **2011**, *29* (4), 341−5.
(76) Melzer, C.; Rehn, V.; Yang, Y.; Bähre, H.; von der Ohe, J.; Hass, R. Taxol-Loaded MSC-Derived Exosomes Provide a Therapeutic Vehicle to Target Metastatic Breast Cancer and Other Carcinoma Cells. *Cancers* **2019**, *11* (6), 798.
(77) Yang, Z.; Shi, J.; Xie, J.; Wang, Y.; Sun, J.; Liu, T.; Zhao, Y.; Zhao, X.; Wang, X.; Ma, Y.; Malkoc, V.; Chiang, C.; Deng, W.; Chen, Y.; Fu, Y.; Kwak, K. J.; Fan, Y.; Kang, C.; Yin, C.; Rhee, J.; Bertani, P.; Otero, J.; Lu, W.; Yun, K.; Lee, A. S.; Jiang, W.; Teng, L.; Kim, B. Y. S.; Lee, L. J. Large-scale generation of functional mRNA-encapsulating exosomes via cellular nanoporation. *Nat. Biomed Eng.* **2020**, *4*, 69.
(78) Limoni, S. K.; Moghadam, M. F.; Moazzeni, S. M.; Gomari, H.; Salimi, F. Engineered Exosomes for Targeted Transfer of siRNA to HER2 Positive Breast Cancer Cells. *Appl. Biochem. Biotechnol.* **2019**, *187* (1), 352−364.
(79) Mern, D. S.; Hasskarl, J.; Burwinkel, B. Inhibition of Id proteins by a peptide aptamer induces cell-cycle arrest and apoptosis in ovarian cancer cells. *Br. J. Cancer* **2010**, *103* (8), 1237−44.
(80) Zhang, K.; Sefah, K.; Tang, L.; Zhao, Z.; Zhu, G.; Ye, M.; Sun, W.; Goodison, S.; Tan, W. A novel aptamer developed for breast cancer cell internalization. *ChemMedChem* **2012**, *7* (1), 79−84.
(81) Ojima, A.; Matsui, T.; Maeda, S.; Takeuchi, M.; Inoue, H.; Higashimoto, Y.; Yamagishi, S. DNA aptamer raised against advanced glycation end products inhibits melanoma growth in nude mice. *Lab. Invest.* **2014**, *94* (4), 422−9.
(82) Lee, Y. J.; Han, S. R.; Kim, N. Y.; Lee, S. H.; Jeong, J. S.; Lee, S. W. An RNA aptamer that binds carcinoembryonic antigen inhibits hepatic metastasis of colon cancer cells in mice. *Gastroenterology* **2012**, *143* (1), 155−65e8.
(83) Nimjee, S. M.; White, R. R.; Becker, R. C.; Sullenger, B. A. Aptamers as Therapeutics. *Annu. Rev. Pharmacol. Toxicol.* **2017**, *57*, 61−79.
(84) Kovacevic, K. D.; Gilbert, J. C.; Jilma, B. Pharmacokinetics, pharmacodynamics and safety of aptamers. *Adv. Drug Delivery Rev.* **2018**, *134*, 36−50.
(85) Weitzel, J. N.; Blazer, K. R.; MacDonald, D. J.; Culver, J. O.; Offit, K. Genetics, genomics, and cancer risk assessment: State of the Art and Future Directions in the Era of Personalized Medicine. *Ca-Cancer J. Clin.* **2011**, *61* (5), 327−59.
(86) Dagogo-Jack, I.; Shaw, A. T. Tumour heterogeneity and resistance to cancer therapies. *Nat. Rev. Clin. Oncol.* **2018**, *15* (2), 81−94.

https://dx.doi.org/10.1021/acssensors.0c02237
ACS Sens. 2021, 6, 1418−1429

(87) McGranahan, N.; Swanton, C. Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. *Cell* **2017**, *168* (4), 613−628.
(88) Vuong, L.; Kotecha, R. R.; Voss, M. H.; Hakimi, A. A. Tumor Microenvironment Dynamics in Clear-Cell Renal Cell Carcinoma. *Cancer Discovery* **2019**, *9* (10), 1349−1357.
(89) Wculek, S. K.; Cueto, F. J.; Mujal, A. M.; Melero, I.; Krummel, M. F.; Sancho, D. Dendritic cells in cancer immunology and immunotherapy. *Nat. Rev. Immunol.* **2020**, *20*, 7.
(90) Leong, S. P.; Tseng, W. W. Micrometastatic cancer cells in lymph nodes, bone marrow, and blood: Clinical significance and biologic implications. *Ca-Cancer J. Clin.* **2014**, *64* (3), 195−206.
(91) Ismail, S. I.; Alshaer, W. Therapeutic aptamers in discovery, preclinical and clinical stages. *Adv. Drug Delivery Rev.* **2018**, *134*, 51−64.
(92) Kaur, H.; Li, J. J.; Bay, B. H.; Yung, L. Y. Investigating the antiproliferative activity of high affinity DNA aptamer on cancer cells. *PLoS One* **2013**, *8* (1), No. e50964.
(93) Roth, F.; De La Fuente, A. C.; Vella, J. L.; Zoso, A.; Inverardi, L.; Serafini, P. Aptamer-mediated blockade of IL4Ralpha triggers apoptosis of MDSCs and limits tumor progression. *Cancer Res.* **2012**, *72* (6), 1373−83.
(94) Zou, J.; Shi, M.; Liu, X.; Jin, C.; Xing, X.; Qiu, L.; Tan, W. Aptamer-Functionalized Exosomes: Elucidating the Cellular Uptake Mechanism and the Potential for Cancer-Targeted Chemotherapy. *Anal. Chem.* **2019**, *91* (3), 2425−2430.
(95) Hu, Y.; Li, D.; Wu, A.; Qiu, X.; Di, W.; Huang, L.; Qiu, L. TWEAK-stimulated macrophages inhibit metastasis of epithelial ovarian cancer via exosomal shuttling of microRNA. *Cancer Lett.* **2017**, *393*, 60−67.
(96) Xie, X.; Nie, H.; Zhou, Y.; Lian, S.; Mei, H.; Lu, Y.; Dong, H.; Li, F.; Li, T.; Li, B.; Wang, J.; Lin, M.; Wang, C.; Shao, J.; Gao, Y.; Chen, J.; Xie, F.; Jia, L. Eliminating blood oncogenic exosomes into the small intestine with aptamer-functionalized nanoparticles. *Nat. Commun.* **2019**, *10* (1), 5476.
(97) Agostinis, P.; Berg, K.; Cengel, K. A.; Foster, T. H.; Girotti, A. W.; Gollnick, S. O.; Hahn, S. M.; Hamblin, M. R.; Juzeniene, A.; Kessel, D.; Korbelik, M.; Moan, J.; Mroz, P.; Nowis, D.; Piette, J.; Wilson, B. C.; Golab, J. Photodynamic therapy of cancer: an update. *Ca-Cancer J. Clin.* **2011**, *61* (4), 250−81.
(98) Chen, Q.; Wen, J.; Li, H.; Xu, Y.; Liu, F.; Sun, S. Recent advances in different modal imaging-guided photothermal therapy. *Biomaterials* **2016**, *106*, 144−66.
(99) Liu, Z.; Lin, H.; Zhao, M.; Dai, C.; Zhang, S.; Peng, W.; Chen, Y. 2D Superparamagnetic Tantalum Carbide Composite MXenes for Efficient Breast-Cancer Theranostics. *Theranostics* **2018**, *8* (6), 1648−1664.
(100) Cai, S.; Yan, J.; Xiong, H.; Liu, Y.; Peng, D.; Liu, Z. Investigations on the interface of nucleic acid aptamers and binding targets. *Analyst* **2018**, *143* (22), 5317−5338.
(101) Kappel, K.; Zhang, K.; Su, Z.; Watkins, A. M.; Kladwang, W.; Li, S.; Pintilie, G.; Topkar, V. V.; Rangan, R.; Zheludev, I. N.; Yesselman, J. D.; Chiu, W.; Das, R. Accelerated cryo-EM-guided determination of three-dimensional RNA-only structures. *Nat. Methods* **2020**, *17* (7), 699−707.
(102) Wan, Y.; Wang, L.; Zhu, C.; Zheng, Q.; Wang, G.; Tong, J.; Fang, Y.; Xia, Y.; Cheng, G.; He, X.; Zheng, S. Y. Aptamer-Conjugated Extracellular Nanovesicles for Targeted Drug Delivery. *Cancer Res.* **2018**, *78* (3), 798−808.
(103) Pi, F.; Binzel, D. W.; Lee, T. J.; Li, Z.; Sun, M.; Rychahou, P.; Li, H.; Haque, F.; Wang, S.; Croce, C. M.; Guo, B.; Evers, B. M.; Guo, P. Nanoparticle orientation to control RNA loading and ligand display on extracellular vesicles for cancer regression. *Nat. Nanotechnol.* **2018**, *13* (1), 82−89.
(104) Xie, X.; Nie, H.; Zhou, Y.; Lian, S.; Mei, H.; Lu, Y.; Dong, H.; Li, F.; Li, T.; Li, B.; Wang, J.; Lin, M.; Wang, C.; Shao, J.; Gao, Y.; Chen, J.; Xie, F.; Jia, L. Eliminating blood oncogenic exosomes into the small intestine with aptamer-functionalized nanoparticles. *Nat. Commun.* **2019**, *10* (1), 5476.

https://dx.doi.org/10.1021/acssensors.0c02237
ACS Sens. 2021, 6, 1418−1429