Biotechnology Journal
www.biotechnology-journal.com

Exosomes for Non-Invasive Cancer Monitoring

*Kalimuthu Kalishwaralal, Woo Young Kwon, and Ki Soo Park**

Exosomes, membrane-bound phospholipid vesicles having diameters of 50–200 nm, are secreted by all cell types and circulate in human body fluids. These vesicles are known to carry cellular constituents that are specific to the originating cells (e.g., cytoplasmic/membrane proteins, RNA, and DNA). Thus, exosomes, which are both structurally stable and abundant, are robust indicators of cancers and, as a result, they have been utilized to monitor this disease in a manner that is less invasive than gold standard tissue biopsies. In this review, the history of exosomes and the specific biomarkers present in exosomes that enable accurate monitoring of various diseases are described. In addition, methods for analysis of exosomes and identification of biomarkers are presented with special emphasis being given to isolation and signaling strategies. Lastly, integrated, microfluidic systems developed for exosome-based cancer diagnosis are described and future directions that research in this area will likely take are presented.

1. Introduction

The identification of biological fluids in humans needed to guide patient management is much less invasive compared to biopsies of organs and tissues.[1] Importantly, exosomes, which are membrane-bound nanovesicles with diameters between 50 and 200 nm, are known to circulate in human body fluids following their secretion by all cell types including B- and T-lymphocytes, neurons, epithelial cells, dendritic cells, and tumor cells.[2] Moreover, exosomes contain molecular markers such as nucleic acids and proteins that are specific to the originating cells (**Figure 1**), and analysis of exosomes can be considered to be a "liquid biopsy" that enables early detection, diagnostic evaluation, effective treatment, and follow-up care of diseased patients in a less-invasive manner.[3]

In the early 2000s, exosomes were found in human bronchoalveolar lavage and urine.[4–7] Since that time, exosomes have been identified in most human body fluids such as blood, saliva, breast milk, and amniotic fluid.[8–15] The interest in exosomes has significantly escalated owing to the fact that they reflect the physiological status of cells from which they originate. It is known that exosomes contain sub-populations of proteins, messenger RNAs (mRNAs), microRNAs (miRNAs), and lipids specifically incorporated during their biogenesis.[16] Notably, specific cellular markers, which are usually present in human body fluids in quantities that are too small to be detected, are enriched in exosomes.[17] The novel features of exosomes have been exploited as sources of clinical information about the presence of various diseases such as cardiovascular diseases, diabetes, infectious diseases, and cancers.[18–21]

Thus far, different types of systems have been developed for exosome analysis, which mainly focus on their isolation from biological fluids and biomarker assays in which signal is generated in proportion to the quantity of each target biomarker.[22,23] In this review, the history of exosomes and specific exosome biomarkers are described. In addition, diagnostic strategies based on exosomes that enable non-invasive cancer monitoring are presented. In particular, a review is given of exosome isolation and signaling methods that are part of streamlined protocols for analysis of exosome biomarkers. Finally, integrated, microfluidic systems developed for exosome-based cancer diagnosis are discussed and the expected directions that future research in this area will follow are presented.

2. History

In 1981, Heine et al. were the first to identify exfoliated vesicles from three cell lines including mouse neuroblastoma, rat glioma, and mouse melanoma, and from two primary cells of rat aorta and mouse astroblast.[24] These workers found that membranes of the isolated vesicles are significantly enriched in sphyngomyelin and polyunsaturated fatty acids, which prompted their conclusion that these vesicles originate from cell membranes. In addition, analysis of the vesicles by using electron microscopy revealed that two types exist, one with a diameter between 500 and 1000 nm and the other with a diameter around 40 nm. Finally, they chose to name these vesicles "exosomes" even though knowledge about their endosomal origin did not exist.[24]

In 1986, Kassis et al. demonstrated the possible physiological role played by exosomes through experiments carried out using exosomes derived from rat glioma C6 cells.[25] The next year, Johnstone et al. proposed a general mechanism for the biogenesis of exosomes involving: 1) endocytosis of specific membrane proteins; 2) formation of MVBs; 3) fusion of their membrane with plasma membranes; and 4) release of their content in the extracellular medium.[26] However, several

Dr. K. Kalishwaralal, W. Y. Kwon, Dr. K. S. Park
Department of Biological Engineering
College of Engineering
Konkuk University
Seoul 05029, Republic of Korea
E-mail: kskonkuk@gmail.com; akdong486@konkuk.ac.kr

DOI: 10.1002/biot.201800430

features of exosomes remained obscure at that time. Specifically, unaddressed questions existed about the cell types and their state at the origin of formation and during maturation.

In the early 1990s, Vingtdeux et al. proposed that the main role played by exosomes is to allow cells to remove non-functional proteins.[27] It was not until the mid-1990s that Raposo et al. demonstrated another role of exosomes in intercellular communication. In particular, these workers discovered that MHC class II compartments in B lymphocytes are localized in late endocytic compartments and that their fusion with the plasma membrane causes release of the vesicles.[28] In addition, they observed that vesicles derived from activated B lymphocytes stimulate T lymphocytes, and that those isolated from bone marrow dendritic cells and then are activated by a tumor peptide play a role in the inhibition of tumor growth.[28,29] The findings suggested for the first time that the nanovesicles have a therapeutic function. Since the publication describing these efforts and because of improved knowledge about the biochemical composition of exosomes, it has become apparent that many cell types, such as B- and T-lymphocytes, neurons, epithelial cells, dendritic cells, and tumor cells, secrete exosomes and that the exosomes secreted by the cells are present in all body fluids including blood, urine, saliva, breast milk, sperm, and amniotic fluid.[30–38]

In order to better understand their biological role(s) and potential as a reservoir of biomarkers, the interest in exosomes has been intensified in the last 15 years and a great effort has been made to characterize exosomes and their contents. A major breakthrough in the analysis of exosomes occurred in 1999 when Thery et al. utilized MALDI-TOF mass spectrometry to identify 11 proteins, including cluster of differentiation 9 (CD9), annexin II, and heat shock cognate 73 (HSC73), that are selectively enriched in the dendritic cell-derived exosomes.[39] In addition, the results of experiments in 2007, performed using exosomes from different cell types and different biological fluids, demonstrated the presence of nucleic acids such as mRNAs and miRNAs in exosomes. Through the use of microarray techniques and radioactive labeling, Valadi et al. observed that exosomes transfer their mRNAs to adjacent cells and that the transferred mRNAs function to produce proteins in the recipient cells. It was also proven that miRNAs are present in exosomes derived from glioblastomas and blood cells, and that regulatory, non-coding RNA exists in exosomes derived from B- and T-lymphocytes, neurons, epithelial cells, dendritic cells, and tumor cells.[40]

Ki Soo Park is an assistant professor in the Department of Biological Engineering at Konkuk University, Korea. He received his PhD from the Department of Chemicaland Biomolecular Engineering from KAIST, Korea. He worked as a post-doctoral researcher in Harvard Medical School/Massachusetts General Hospital. His research interests include exosome analysis platforms, nucleic acid bioengineering, nano-biotechnology for biomedical sensing, and point-of-care diagnostic systems.

3. Exosome as a Biomarker Reservoir

The involvement of exosomes in immunity and intercellular communication suggests that they possess enormous potential for human pathology as reservoirs of diagnostic and prognostic biomarkers.[41] A number of experimental investigations have demonstrated that exosomes in biological fluids contain a cargo of nucleic acids, proteins, and lipids, including those that are also expressed by their parent cells.[42] Owing to their structural stability and abundance, exosomes serve as surrogates of their parental cells in non-invasive methods for monitoring various biological processes including the development of genetic diseases and cancers, and aging.[43]

3.1. Exosome RNAs

Most nucleic acids in biological fluids are susceptible to degradation by extracellular RNases or DNases because they are not sheltered in the plasma membrane following secretion from the original cells. However, two exceptions to this situation exist.[44] The first is that extracellular miRNAs are protected from degradation by association with either nucleophosmin or argonaute protein.[42] The second is that exosome RNAs encapsulated inside the phospholipid bilayers are protected from degradation. Observations made in many studies have shown that exosome RNAs are stable and intact in most biological fluids such as saliva, urine, and blood.[44]

One example demonstrating the presence of exosome RNAs is found in studies investigating the roles of exosomes in maternity. The results of this effort showed that placental-specific exosome miRNA passes into the circulating blood of the

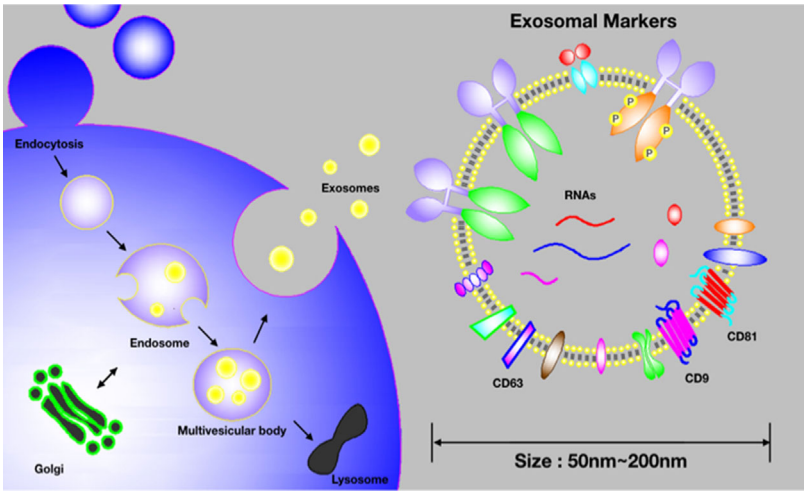

Figure 1. Illustration of exosome biogenesis and their biomarkers.

Table 1. Exosome RNA biomarkers.

Cancer type	Exosome miRNA	Exosome mRNA	Exosome circRNA	Exosome lncRNA	References
Colorectal cancer	miR-17-92a, miR-19a, let-7a, miR-1229, miR-1246, miR-150, miR-223, miR-23a, miR-193a, miR148, miR126, miR-210	KRTAP5–4, MAGEA3	CDR1as, CircHIPK3, TUG1, Circ-KLDHC10, CircFAT1, CircRTN4	BCAR4, CRNDE-h, ZFAS1,	[121–131]
Breast cancer	miR-24-3p, miR-891a, miR-106a-5p, miR-20a-5p, miR-1908, miR-101, miR-372, miR-373, miR-21, miR-1246	RAB13, RPPH1, EEF1A1, FTH1, FTL, RPL28	–	UCA1	[138–141]
Lung cancer	miR-19b-1, miR-1285, miR-1289, miR-1303, miR-217, miR-29a-5p, miR-548-3p, miR-650, miR-21, miR-425	ZEB1	Circ_0013958	MALAT-1	[103–108]
Pancreatic cancer	miR-1246, miR-4644, miR-3976, miR-4306, miR-17-5p, miR-21	Mutated KRAS	Circ_IRAS	Sox2ot	[50,132–135]
Prostate cancer	miR-200c, miR-605, miR-135a, miR-433, miR-106a, miR141, miR-375	hTERT, PCA3, T2E	CircSMARCA5	PCA3, lincRNA-p21	[47,113–120]
Glioblastoma	miR-320, miR-574-3p	EGFRvIII	Circ-TTBK2	HOTAIR	[109–112]
Ovarian cancer	miR-21, miR-141, miR-200a, miR-200c, miR-200b, miR-203, miR-205, miR-214	–	–	–	[50]
Esophageal cancer	miR-21	–	–	–	[142]
Osteosarcoma	–	Annexin 2, Smad2, P27, MTAP, CIP4, PEDF	Circ_0004674	–	[136,137]
Cervical cancer	mir-21, mir-146a	–	–	HOTAIR, MALAT1, MEG3A	[123,143]
Bladder cancer	–	–	–	MEG3, SNHG16, HOTAIR, HOXA-AS2, MALAT-1, lincRoR, HYMA1, linc00477, LOC100506688, OTX2-AS1	[144,145]

mother where their levels remain constant and disappear only after birth of the baby.[45] Another example is found in research executed utilizing different types of cancers (**Table 1**). The results demonstrated that exosomes derived from prostate cancer patients contain 7 specific miRNAs, including miR-200c, miR-605, and miR-135a, which are potential biomarkers for the diagnosis of this cancer type.[46] In addition, they showed that specific mRNAs, such as prostate cancer associated 3 (PCA3) and TMPRSS2:ERG (T2E), are present in exosomes derived from the urine of prostate cancer patients.[47] The second key observation arose from studies of lung cancer patients. It was found that 11 specific miRNAs, such as miR-19b-1, miR-1285, and miR-1289, are present in the circulating exosomes of these patients and that they can serve as potential markers for the diagnosis of this disease.[48] The third concerns esophageal cancer, whose evolution was found to correlate with miR-21 in exosomes.[49] Fourthly, it was demonstrated that 8 specific miRNAs, such as miR-21, miR-141, and miR-200a, are expressed in ovarian cancer patients and that these markers can be used for diagnosis of the disease.[50] Especially important is the finding that the two miRNAs, miR-200c and miR-214, are specific for monitoring the progression of this ovarian cancer because they are only poorly expressed in exosomes of patients with low-grade lesions but strongly expressed in patients with stage 4 ovarian cancer.[50] The last example concerns studies of glioblastomas, which found that patient-derived exosomes contain miRNAs such as miR-320 and miR-574-3p. In addition, 16 exosome miRNAs, such as miR-200, miR-10b, miR-21, and miR-105, are present in increased amounts in patients with glioblastomas that have undergone metastasis, suggesting that these miRNAs could serve as prognostic markers.[51] Overall, a number of exosome RNAs, especially miRNAs specific to different cancers, have been identified and extensively utilized for the diagnosis of different cancers. In the same manner, other types of RNAs such as mRNA, circRNAs, and lncRNAs, which have been found in exosomes, have been also utilized for the diagnosis of different cancers (Table 1).

3.2. Exosome Proteins

Several studies have been performed to identify exosome proteins in different biological fluids. In 2007, Admyre et al. isolated nanovesicles from maternal milk and, by using mass spectrometry and Western blotting, they identified several exosome protein markers including human leukocyte antigen–antigen D related (HLA-DR), HSC70, and CD81.[52] In a similar manner, Gonzalez-Begne et al. characterized 491

Table 2. Exosome protein markers

Cancer type	Exosome protein markers	References
Colorectal cancer	CEA, CD147, and GPC1	[146–148]
Breast cancer	MUC1, Survivin, Survivin-2B, CEA, CA 15-3, CD38, SLCO2A1, MTDH, FGFR, and TGFBR1	[149–153]
Lung cancer	CD151, TSPAN8, CD171, EpCAM, EGFR, CEA, and LRG1	[154–158]
Pancreatic cancer	GPC1, MIF, RAB27A, and TP53	[159,160]
Prostate cancer	Flot2, TMEM256, Rab3B, LAMTOR1, FABP5, GGT, T2E, β-catenin, PCA3, PSA, PSMA, ITGA3, ITGB1, survivin, and PSE	[47,110,120,121,122,161]
Glioblastoma	EGFR, EGFRvIII, and CD63	[110,113]
Ovarian cancer	CLDN4, L1CAM, CD24, ADAM10, CD147, TGFβ1, MAGE3/6, CA-125, and EpCAM	[162–168]
Bladder cancer	A1AT, H2B1K, EGF, Gs alpha subunit, resistin, RAI3, TACSTD2, EDIL3, MUC4, EPS8L2, ITGA6, MUC1, and CD147	[169–171]
Melanoma	TYRP2, VLA-4, HSP70, HSP90 isoform, CD63, Cav1, HAPLN1, GRP78, SDCBP, ANXA1, and ANXA2	[172,173]
Renal cell cancer	MMP-9, CD147, and CA IX	[169,174]
Stomach cancer	HER2 and CCR6	[169,175]

exosome proteinsin saliva, and Poliakov et al. and Choi et al. determined the identities of 440 proteins in the exosomes of prostate cancer patients and 846 proteins in those of colorectal cancer patients, respectively.[53–55]

Many attempts have been made to identify cancer-specific exosome protein markers (**Table 2**). Representative examples of successes in these searches are found in experiments performed with prostate cancer patients, which led to the discovery that exosomes from this source contain increased amounts of protein markers, such as Ephrin type-A receptor 2 (EphA2) and CD276, when premature senescence is induced by UV irradiation.[56] These results indicate that EphA2 and CD276 could play roles as prognostic biomarkers for the evaluation of the effectiveness of cancer treatments, in response to which they are up-regulated.[56] In addition, blood exosomes of the ovarian cancer patients were found to contain the specific protein, claudin-4 (CLDN4), and its expression in these vesicles was found to correlate with the presence of cancer antigen 125 (CA125), the single tumor marker used for diagnosis and management of ovarian cancers.[57,58] Recently, the cell surface proteoglycan, glypican-1 (GPC1), was found to be specifically enriched on cancer cell-derived exosomes and successfully used to detect patients with pancreatic cancer in both a highly specific and sensitive manner.[59] Overall, the results clearly demonstrate the potential utility of exosomes for the accurate diagnosis of various cancers.

3.3. Exosome Lipids

The lipids, a major component of plasma membrane, are also one of the exosome markers. Several studies have shown that the lipids such as cholesterol, sphingolipids, glycosphingolipids, and phospholipids are enriched in exosomes.[60] Specifically, it was found that exosomes secreted from the prostate cancer, PC-3 cell line, possess approximately 280 lipid species from 18 lipid classes.[61] In addition, the recent studies executed with 15 prostate cancer patients and 13 healthy controls, clearly demonstrated that the level of sphingolipids are substantially higher in the prostate cancer patients than in the healthy controls.[62] Overall, these results confirm that the exosome lipids like nucleic acids and proteins can serve as the biomarker for the diagnosis of cancers.[63]

4. Exosome Isolation Methods

The first step in deriving useful clinical information from exosomes involves their isolation from biological fluids.[64] Thus far, several isolation methods, which take advantage of the unique structural and biochemical properties of exosomes, have been utilized for this purpose including ultracentrifugation, density gradient centrifugation, filtration, chemical precipitation, and immunoaffinity capture-based techniques (**Table 3**).[65] In this section, we present an overview of exosome isolation methods with an emphasis being given to protocols and challenges.[64,65]

4.1. Ultracentrifugation

Ultracentrifugation is a conventional yet still widely employed method to isolate exosomes. In this process, centrifugation at a relatively low speed ($<10\,000g$) is initially utilized to remove sediment and other cellular debris. This step is followed by centrifugation at $200\,000g$ to separate exosomes from the supernatant. Although the overall protocol enables effective isolation of extracellular vesicles, it requires a relatively long time period.[66] A more serious drawback is that the purity of the isolated exosomes is typically so poor that contaminate proteins coming from other sources are present in the isolates and

Table 3. Exosome isolation methods

Techniques	Advantages	Disadvantages	References
Ultracentrifugation	- Low cost - Large sample volume	- Bulky instrument - Long preparation time - Low purity and low reproducibility - Damaged exosomes - Not compatible with high-throughput analysis	[176]
Density gradient centrifugation	- High purity	- Bulky instrument - Long preparation time - Not compatible with high-throughput analysis	[177]
Filtration	- Simple procedure - Rapidity - High-throughput analysis	- Filter plugging - Low purity (protein contamination)	[178]
Chemical precipitation	- Simple procedure - High integrity - No bulky instrument	- Low purity (contamination and retention of the precipitant) - Long preparation time	[179,180]
Immunoaffinity-capture	- High purity - High selectivity	- High cost (requirement of available antibodies) - Difficulties in removing the antibodies and obtaining the intact vesicles (eluting buffers can damage exosomes) - Not compatible with high-throughput analysis	[180]

interfere with accurate analysis.[67] For example, the most abundant glycoprotein in urine, Tamm–Horsfall protein (THP), was observed to be present in large quantities in exosomes isolated by ultracentrifugation.[68] Despite being plagued by this issue, the centrifugation method is still used in most clinical laboratories because it is relatively easy to perform and it enables processing of a large sample volume.[69]

4.2. Density Gradient Centrifugation

To minimize non-relevant protein contamination arising from application of the ultracentrifugation protocol, another method that relies on sucrose density gradient centrifugation was developed.[70] The first step in this method involves depositing precipitates arising from centrifugation at 150 000*g* on top of a gradient of 5–30% sucrose followed by centrifugation at 200 000*g*. Finally, each produced fraction is diluted and centrifuged at 150 000*g*.[70] It was found that exosomes are enriched at a buoyant density of 1.11 g mL^{-1}, which is consistent with that reported for exosomes isolated from a diverse range of cell types.[72] As a result, a homogenous population of exosomes that have a size distribution in the range 50–200 nm is obtained.[71,72] However, this method requires multiple centrifugation steps and associated long time periods and it is not compatible with high-throughput analysis.[70]

4.3. Filtration

In order to isolate exosomes in simple and fast manner, several research groups have carried out studies aimed at developing methods that are based on filtration. In this procedure, the supernatant produced by centrifugation of the biological fluid at 17 000*g* is deposited on nanomembrane filter with an average pore diameter of 13 nm, and then centrifuged at 3000*g*.[73] As compared to the ultracentrifugation and density gradient centrifugation methods, this protocol requires a total isolation time of less than 1 h. However, this rapid method leads to low exosome purities caused by contamination with large proteins and other extracellular vesicles.[74] This drawback becomes a particularly serious problem in the diagnosis of renal diseases such as proteinuria, in which numerous proteins are present in the urine of diseased patients.[74]

4.4. Chemical Precipitation

An attempt has been made to isolate exosomes without employing an ultracentrifugation step. This aim has led to the development of the chemical precipitation method, in which chemical components such as polyethylene glycol (PEG) are utilized to exclude water, which drives exosomes out of solution so that they can be separated by using low-speed centrifugation.[75] This method is easily performed and does not require expensive instrumentation, but it does need overnight incubation. In addition, in this approach it is not possible to completely separate the chemical precipitant from exosomes and the target exosomes are often contaminated with unwanted vesicles, which affects accurate analysis of the target exosomes and leads to misinterpretations of the findings.[76]

4.5. Immunoaffinity Capture

Because the membranes of exosomes contain large quantities of proteins and receptors, the possibility exists that exosomes can be isolated by taking advantage of specific immunoaffinity interactions between antigens and antibodies. It is known that CD9, CD63, and CD81, which are members of the tetraspanin family, are present in abundance on membranes of most human exosomes and, thus, they have been used as pan-exosome

markers.[77,78] In addition, specific tumor markers such as epidermal growth factor receptor (EGFR) and epithelial cellular adhesion molecule (EpCAM) have been targeted in methods to isolate tumor-specific exosomes and to analyze subpopulations of exosomes.[79] Generally in this procedure, antibodies specific to exosome markers are incorporated on a solid support, such as magnetic beads, to capture exosomes through antigen-antibody interactions. The exosome containing support is then separated by using one of a number of different support-selective procedures. This method for isolation of exosomes from colon cancers was shown to be superior to those involving ultracentrifugation and density gradient ultracentrifugation.[78] Despite the numerous advantages held by immunoaffinity-capture based methods such as rapidity and easiness, they have critical limitations associated with the fact that expression of specific markers varies between cell types because of the heterogeneity of tumors. This variation can lead to different isolation efficiencies, which hinders accurate and reproducible exosome analysis.[80]

5. Exosome Signaling Methods

Effective signal generation methods are required for accurate identification of specific biomarkers that are highly expressed on the isolated exosomes. Until now, various signaling methods, including surface plasmon resonance, electrochemistry, colorimetry, and fluorometry, have been employed for this purpose.[81–83] In the following section, key methods for the detection of specific exosome markers developed using these approaches are presented with emphasis given to their major components and techniques used.

5.1. Surface Plasmon Resonance

A representative example for exosome protein analysis is found in recent studies by Im et al.,[84] in which a nano-plasmonic exosome (nPLEX) assay, was developed. The assay relies on optical transmission through periodic nanoholes rather than total internal reflection, which is used in commercial surface plasmon resonance systems. In the system, spectral shifts of resonant light transmission proportional to the levels of a target marker occur only when specific exosomes are captured on periodic nanoholes through antigen-antibody interactions. This method enables label-free, sensitive, and multiplexed analysis of exosome markers. In preliminary studies, Im et al. confirmed the efficacy of the nPLEX system by demonstrating its application to the analysis of ovarian cancer exosomes, the results of which show that these exosomes highly express two markers, EpCAM and CD24, providing a diagnostic accuracy of 97%. This approach has a large potential for use in the analysis of target exosome markers (e.g., protein, mRNA, miRNA, or DNA) in a high-throughput manner. In this regard, Im et al. devised the first version of an automated nPLEX system that contains over 100 sensing locations to carry out analyses of clinical samples. This system was employed to screen exosome markers for pancreatic cancers and found a combination of pancreatic cancer specific markers (EGFR, EpCAM, Mucin-1 (MUC1), wingless-type MMTV integration site family, member 2 (WNT2), and GPC1), which were utilized to diagnose 135 patients undergoing surgery for pancreatic pathologies.[85]

5.2. Electrochemistry

Electrochemical sensing has unique features associated with high sensitivity, portability, operational simplicity, and low-power consumption. This method has many significant benefits, including exosome isolation and detection in a single device platform, and more affordable multiplexed screening. As a result, the electrochemical method has been utilized in system developed for the analysis of exosome markers. In one study, Jeong et al. devised a compact electrochemical system, called an integrated magneto-electrochemical assay (iMEX), for rapid, on-site screening of exosomes.[86] In this sensor, exosomes are captured on magnetic beads through antigen–antibody interactions and specific exosome markers are identified by the presence of characteristic electrochemical signals amplified by the enzyme-conjugated antibodies bound onto the exosome. The efficacy of this system was validated using exosomes derived from ovarian cancer patients. The results of electrochemical analysis confirmed that expression of the two markers, EpCAM and CD24, is higher in ovarian cancer patients than in healthy controls. The utility of this method was further demonstrated by its use in the analysis of exosomes from ovarian cancer patients undergoing drug treatment.[86] In addition, the electrochemical assay protocol, which outperforms traditional approaches like ELISA and Western blotting, has been applied to probe urinary exosomes for the detection of kidney transplant rejection. Based on the assumption that the exosomes released by immune cells into urine can serve as surrogate biomarker for kidney transplant rejection, a screen was conducted to identify markers specific to the immune cell-derived exosomes. The results show that CD3 is the most effective marker for distinguishing patients that have a high propensity to reject transplant from non-rejection patients.[87]

5.3. Colorimetry

Colorimetric sensing, which can be carried out with the naked eye, has been applied along with nanozymes, nanomaterials with enzyme-like characteristics, in designing systems for the diagnosis of exosome markers. A representative example of this approach is found in a colorimetric sensing strategy for analysis of exosomes, which takes advantage of the peroxidase-mimicking activity of nanomaterials such as single-walled carbon nanotubes and graphitic carbon nitride nanosheets.[83,88] In this assay system, DNA aptamers specific to the exosome surface protein, CD63, are adsorbed on the surface of the nanomaterial, significantly enhancing its catalytic activity, and associated intense colorimetric response.[88] In contrast, the presence of exosomes that express CD63 causes detachment of DNA aptamers from the nanomaterial, resulting in a reduced catalytic activity and a significant decrease in the colorimetric signal, which can be easily detected using the naked eye. The developed colorimetric sensors are simple to use, do not require

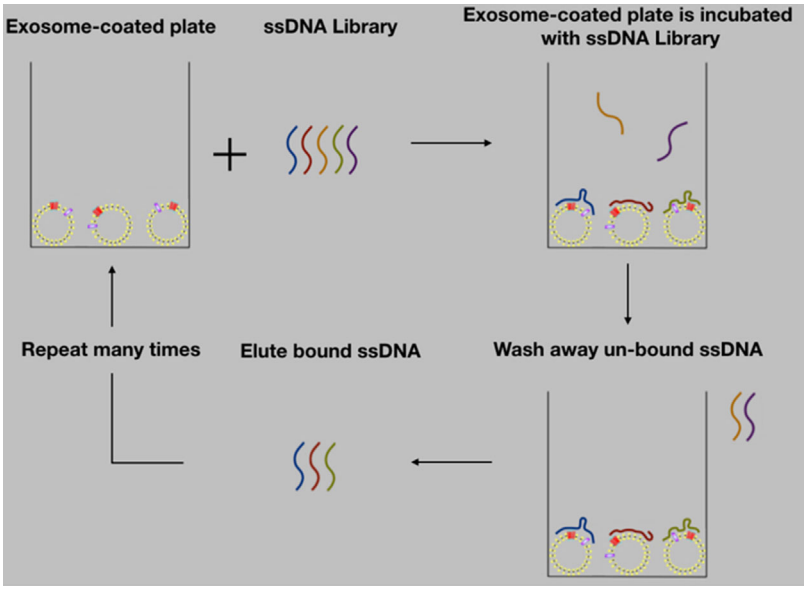

Figure 2. Aptamer technique to screen exosome biomarkers.

washing steps and can be universally applied for the detection of target markers.

6. Conclusion and Future Directions

The growing emphasis on targeted and personalized therapies has led to an increased need for biomarkers that can be utilized to diagnose cancers accurately and to predict drug sensitivities effectively, both of which are crucial for guiding treatment decisions.[89] While archival tumor biopsies provide valuable initial information about potential therapeutic targets, they often do not generate results that correlate with pathways of activation occurring at the time of patient enrollment.[90] As a result, this approach under-values the dynamic nature of protein modification and tumor evolution during therapy. On the other hand, more informative serial tumor biopsies are prohibitively invasive and costly.[91] A promising new approach to cancer diagnosis exploits circulating biomarkers that can be repeatedly and conveniently assessed with minimal complications. This is especially true of circulating exosomes, which contain a number of biomarkers specific to the originating cells.[92] As a result, exosome analysis has garnered increasing attention owing to its novel features including the fact that exosomes function as reliable surrogates of parental cells and that they are both abundant and structurally stable. Thus, the contents of exosomes better reflect overall tumor burdens and, as a result, their analysis avoids limitations of tumor heterogeneity and sampling bias.[93]

Thus far, antibodies have been widely utilized for specific recognition of exosome biomarkers. For example, Li et al. developed a novel immunoassay for the early diagnosis of pancreatic cancer, which relies on the surface-enhanced raman scattering with ultrathin polydopamine-encapsulated reporter.[94] However, this approach is critically limited by factors such as instability, high-cost, and clonal variations of antibodies. More seriously, if no information exists about specific exosome markers or validated antibodies, the detection of cancers by analyzing exosomes is not possible.[95] Thus, there currently exists a high demand for the methods to diagnose specific cancers in the absence of knowledge about biomarkers. Toward this goal, in vitro selection procedure called Systematic Evolution of Ligands by EXponential enrichment (SELEX) introduced in 1990s has received special attention. In this system, a large random DNA libraries that consist of as many as 10^{14} single-strand DNAs interact with a target molecule.[96] Through an iterative process, ssDNA that has the highest affinity and selectivity for a target molecule, which is an aptamer, is created. Since the time of its development, the aptamer technology has been used as a high-throughput screening tool in many areas such as immunology, cell biology, molecular imaging, and pharmacology.[97] In recent years, this technique has been applied to uncover protein receptors that are specific to markers over-expressed in different cancers.[98] We feel that this technique has the potential to be employed to analyze exosomes derived from different cancers. Once developed, this method should enable early diagnosis of specific cancers in the absence of any prior knowledge about biomarkers.[99] Consequently, the diagnostic and prognostic value of exosomes will be enhanced (**Figure 2**).

In recent years, a large effort has been also given to develop effective procedures for exosome isolation and analysis. Microfluidics-based technologies that integrate exosome isolation and detection in a single platform have been suggested to be the basis of a promising approach for carrying out fast and high-throughput analysis of exosome markers.[100] In recent years, Xu et al., reported two-stage microfluidic platform (ExoPCD-chip) which integrates on-chip isolation and in-situ electrochemical analysis of exosomes. The developed system was utilized for the diagnosis of liver cancer patients.[101] In addition, critical are methods to screen panels of exosome-derived proteins, their post-translational modifications and RNA targets. To reach this goal, comprehensive profiling systems are required for simultaneous and rapid analysis of exosome proteins and nucleic acids using a single platform.[102] Investigations focusing on these issues should enable full exploitation of the information contained within exosomes and, consequently, accurate diagnosis of cancers and effective prediction of therapies.

Abbreviations

ABCB10, ATP binding cassette subfamily B member 10; A1AT, alpha-1 antitrypsin; ADAM10, A disintegrin and metalloproteinase domain-containing protein 10; ANXA1, annexin A1; ANXA2, annexin A2; BCAR4, breast cancer anti-estrogen resistance 4; CA 15-3, cancer antigen 15-3; CA IX, carbonic anhydrase IX; CA125, cancer antigen 125; Cav1, caveolin-1; CCR6, chemokine receptor type 6; CD3, cluster of differentiation 3; CD9, cluster of differentiation 9; CD24, cluster of differentiation 24; CD38, cluster of differentiation 38; CD63, cluster of differentiation 63; CD147, cluster of differentiation 147; CD151, cluster of differentiation 151;

CD171, cluster of differentiation 171; CD276, cluster of differentiation 276; CEA, carcinoembryonic antigen; CIP4, Cdc42-interacting protein 4; CLDN4, claudin-4; CRNDE, colorectal neoplasia differentially expressed; EDIL3, EGF-like repeats and discoidin domains 3; EEF1A1, eukaryotic translation elongation factor 1 alpha 1; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EGFRvIII, epidermal growth factor receptor variant III; EpCAM, epithelial cellular adhesion molecule; EphA2, ephrin type-A receptor 2; EPS8L2, epidermal growth factor receptor kinase substrate 8-like protein 2; FABP5, fatty acid-binding protein 5; FGFR, fibroblast growth factor receptor; Flot2, flotillin-2; FTH1, ferritin heavy chain 1; FTL, ferritin light chain; FUT8, fucosyltransferase 8; GGT, gamma-glutamyl transferase; GPC1, glypican-1; GRP78, glucose-regulated protein 78; H2B1K, histone H2B type 1K; HAPLN1, hyaluronan proteoglycan link protein 1; HER2, human epidermal growth factor receptor 2; HIPK3, homeodomain interacting protein kinase 3; HLA-DR, human leukocyte antigen-antigen D related; HOXA-AS2, HOXA cluster antisense RNA 2; HSC, heat shock cognate; HSP70, heat shock protein 70; HSP90 isoform, heat shock protein 90 isoform; hTERT, human telomerase reverse transcriptase; HYMA1, hydatidiform mole associated and imprinted non-protein coding RNA 1; iMEX, integrated magneto-electrochemical assay; ITGA3, integrin alpha 3; ITGA6, integrin alpha 6; ITGB1, integrin beta 1; KRTAP5-4, keratin associated protein 5-4, L1CAM, L1 cell adhesion molecule; LAMTOR1, late endosomal/lysosomal adaptor, MAPK and MTOR activator 1; LRG1, leucine-rich alpha-2-glycoprotein-1; MAGE3/6, melanoma antigen gene 3/6; MALAT-1, metastasis-associated lung adenocarcinoma transcript 1; MEG3, maternally expressed 3, MIF, macrophage migration inhibitory factor; miRNAs, MicroRNAs; MMP9, matrix metalloproteinase-9; mRNAs, messenger RNAs; MTDH, metadherin; MTAP, S-methyl-5′-thioadenosine phosphorylase; MUC1, mucin-1; MUC4, mucin-4; MVBs, multivesicular bodies; nPLEX, nano-plasmonic exosome; OTX2-AS1, OTX2 antisense RNA 1; PCA3, prostate cancer antigen 3; PEDF, pigment epithelium-derived factor, PEG, polyethylene glycol; PSA, prostate-specific antigen; PSE, prostate-specific Ets; PSMA, prostate-specific membrane antigen; RAI3, retinoic acid-induced protein 3; RPPH1, ribonuclease P RNA component H1; RPL28, ribosomal protein L28; RTN4, reticulon-4; SDCBP, syndecan binding protein; Smad2, mothers against decapentaplegic homolog 2; SELEX, systematic evolution of ligands by exponential enrichment; SLCO2A1, solute carrier organic anion transporter family member 2A1; SNHG16, small nucleolar RNA host gene 16; T2E, TMPRSS2:ERG (transmembrane protease, serine 2:ETS-related gene); TACSTD2, tumor-associated calcium signal transducer 2; TCF25, transcription factor 25; TGFBR1, transforming growth factor beta receptor 1; TGFβ1, transforming growth factor beta-1; THP, Tamm–Horsfall protein; TMEM256, transmembrane protein 256; TP53, tumor protein p53; TSPAN8, tetraspanin 8; TTBK2, tau-tubulin kinase 2; TUG1, taurine up-regulated 1; TYPR2, tyrosinase-related protein-2; VLA-4, very late activation antigen-4; WNT2, wingless-type MMTV integration site family member 2; ZEB1, zinc finger E-box binding homeobox 1.

Acknowledgement

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (No. 2018R1D1A1B07050079).

Conflict of Interest

The authors declare no financial or commercial conflict of interest.

Keywords

biosensor, cancer diagnosis, exosomes, isolation, liquid biopsy

Received: July 19, 2018
Revised: October 2, 2018
Published online: November 20, 2018

[1] A. Di Meo, J. Bartlett, Y. Cheng, M. D. Pasic, G. M. Yousef, *Mol. Cancer* **2017**, *16*, 80.
[2] M. Colombo, G. Raposo, C. Théry, *Annu. Rev. Cell Dev. Biol*. **2014**, *30*, 255.
[3] G. Brock, E. Castellanos-Rizaldos, L. Hu, C. Coticchia, J. Skog, *Transl. Cancer Res*. **2015**, *4*, 280.
[4] M. P. Caby, D. Lankar, C. Vincendeau-Scherrer, G. Raposo, C. Bonnerot, *Int. Immunol*. **2005**, *17*, 879.
[5] T. Pisitkun, R. F. Shen, M. A. Knepper, *Proc. Natl. Acad. Sci*. **2004**, *101*, 13368.
[6] C. Admyre, J. Grunewald, J. Thyberg, S. Gripenback, G. Tornling, A. Eklund, A. Scheynius, S. Gabrielsson, *Eur. Respir. J*. **2003**, *22*, 578.
[7] K. R. Qazi, P. Torregrosa, B. Paredes, J. Dahlberg, A. Eklund, S. Gabrielsson, *Thorax* **2010**, *65*, 1016.
[8] M. P. Caby, D. Lankar, C. Vincendeau-Scherrer, G. Raposo, C. Bonnerot, *Int. Immunol*. **2005**, *17*, 879.
[9] S. Nair, K. D. Tang, L. Kenny, C. Punyadeera, *Oral Oncol*. **2018**, *84*, 31.
[10] F. Andre, N. E. C. Schartz, M. Movassagh, C. Flament, P. Pautier, P. Morice, C. Pomel, C. Lhomme, B. Escudier, T. Le Chevalier, T. Tursz, S. Amigorena, G. Raposo, E. Angevin, L. Zitvogel, *Lancet* **2002**, *360*, 295.
[11] K. Skriner, K. Adolph, P. R. Jungblut, G. R. Burmester, *Arthritis Rheum*. **2006**, *54*, 3809.
[12] C. Admyre, S. M. Johansson, K. R. Qazi, J. J. Filen, R. Lahesmaa, M. Norman, E. P. Neve, A. Scheynius, S. Gabrielsson, *J. Immunol*. **2007**, *179*.
[13] C. Admyre, J. Grunewald, J. Thyberg, S. Gripenback, G. Tornling, A. Eklund, A. Scheynius, S. Gabrielsson, *Eur. Respir. J*. **2003**, *22*, 578.
[14] J. L. Gatti, S. Métayer, M. Belghazi, F. Dacheux, J. L. Dacheux, *Biol. Reprod*. **2005**, *72*, 1452.
[15] Y. Ogawa, M. Kanai-Azuma, Y. Akimoto, H. Kawakami, R. Yanoshita, *Biol. Pharm. Bull*. **2008**, *31*, 1059.
[16] M. Riazifar, E. J. Pone, J. Lötvall, W. Zhao, *Annu. Rev. Pharmacol*. **2017**, *57*, 125.
[17] S. Boukouris, S. Mathivanan, *Proteom. Clin. Appl*. **2015**, *9*, 358.
[18] C. Lawson, J. M. Vicencio, D. M. Yellon, S. M. Davidson, *J. Endocrinol*. **2016**, *228*, 57.
[19] Z. Z. Jiang, Y. M. Liu, X. Niu, J. Y. Yin, B. Hu, S. C. Guo, Y. Fan, Y. Wang, N. S. Wang, *Stem Cell Res. Ther*. **2016**, *7*, 24.
[20] G. Coakley, R. M. Maizels, A. H. Buck, *Trends Parasitol*. **2015**, *31*, 477.
[21] C. Sheridan, *Nat. Biotechnol*. **2016**, *34*, 359.
[22] B. S. Chia, Y. P. Low, Q. Wang, P. Li, Z. Gao, *TrAC Trends Anal. Chem*. **2017**, *1*, 93.
[23] L. Wu, X. Qu, *Chem. Soc. Rev*. **2015**, *44*, 2963.
[24] E. G. Trams, C. J. Lauter, J. N. Salem, U. Heine, *Biochim. Biophy. Acta* **1981**, *645*, 63.
[25] S. Kassis, C. J. Lauter, M. Stojanov, N. Salem, *Biochim. Biophys. Acta* **1986**, *886*, 474.
[26] R. M. Johnstone, M. Adam, J. R. Hammond, L. Orr, C. Turbide, *J. Biol. Chem*. **1987**, *262*, 9412.
[27] V. Vingtdeux, N. Sergeant, L. Buée, *Front. Physiol*. **2012**, *3*, 229.
[28] G. Raposo, H. W. Nijman, W. Stoorvogel, R. Liejendekker, C. V. Harding, C. J. Melief, H. J. Geuze, *J. Exp. Med*. **1996**, *183*, 1161.
[29] L. Zitvogel, A. Regnault, A. Lozier, J. Wolfers, C. Flament, D. Tenza, P. Ricciardi-Castagnoli, G. Raposo, S. Amigorena, *Nat. Med*. **1998**, *4*, 594.

[30] G. Van Niel, G. Raposo, C. Candalh, M. Boussac, R. Hershberg, N. Cerf-Bensussan, M. Heyman, *Gastroenterology* **2001**, *121*, 337.
[31] J. Fauré, G. Lachenal, M. Court, J. Hirrlinger, C. Chatellard-Causse, B. Blot, J. Grange, G. Schoehn, Y. Goldberg, V. Boyer, F. Kirchhoff, G. Raposo, J. Garin, R. Sadoul, *Mol. Cell. Neurosci*. **2006**, *31*, 642.
[32] B. Fevrier, D. Vilette, F. Archer, D. Loew, W. Faigle, M. Vidal, H. Laude, *Proc. Natl. Acad. Sci*. **2004**, *101*, 9683.
[33] M. P. Caby, D. Lankar, C. Vincendeau-Scherrer, G. Raposo, C. Bonnerot, *Int. Immunol*. **2005**, *17*, 879.
[34] T. Pisitkun, R. F. Shen, M. A. Knepper, *Proc. Natl. Acad. Sci*. **2004**, *101*, 13368.
[35] Y. Ogawa, Y. Miura, A. Harazono, M. Kanai-Azuma, Y. Akimoto, H. Kawakami, T. Yamaguchi, T. Toda, T. Endo, M. Tsubuki, R. Yanoshita, *Biol. Pharm. Bull*. **2011**, *34*, 13.
[36] C. Admyre, J. Grunewald, J. Thyberg, S. Gripenbäck, G. Tornling, A. Eklund, A. Scheynius, S. Gabrielsson, *Eur. Respir. J*. **2003**, *22*, 578.
[37] G. Ronquist, I. Brody, *Biochim. Biophys. Acta* **1985**, *822*, 203.
[38] A. Asea, C. Jean-Pierre, P. Kaur, P. Rao, I. M. Linhares, D. Skupski, S. S. Witkin, *J. Reprod. Immunol*. **2008**, *79*, 12.
[39] C. Théry, A. Regnault, J. Garin, J. Wolfers, L. Zitvogel, P. Ricciardi-Castagnoli, G. Raposo, S. Amigorena, *J. Cell Biol*. **1999**, *147*, 599.
[40] H. Valadi, K. Ekström, A. Bossios, M. Sjöstrand, J. J. Lee, J. O. Lötvall, *Nat. Cell Biol*. **2007**, *9*, 654.
[41] J. De Toro, L. Herschlik, C. Waldner, C. Mongini, *Front. Immunol*. **2015**, *4*, 203.
[42] A. V. Vlassov, S. Magdaleno, R. Setterquist, R. Conrad, *Biochim. Biophys. Acta* **2012**, *1820*, 940.
[43] R. Xu, A. Rai, M. Chen, W. Suwakulsiri, D. W. Greening, R. J. Simpson, *Nat. Rev. Clin. Oncol*. **2018**, *15*, 617.
[44] A. Beach, H. G. Zhang, M. Z. Ratajczak, S. S. Kakar, *J. Ovarian Res*. **2014**, *7*, 14.
[45] Y. Ouyang, J. F. Mouillet, C. B. Coyne, Y. Sadovsky, *Placenta* **2014**, *35*, 69.
[46] Y. Peng, C. M. Croce, *Signal Transduct. Target. Ther*. **2016**, *28*, 15004.
[47] J. Nilsson, J. Skog, A. Nordstrand, V. Baranov, L. Mincheva-Nilsson, X. O. Breakefield, A. Widmark, *Br. J. Cancer* **2009**, *100*, 1603.
[48] T. Sun, B. Kalionis, G. Lv, S. Xia, W. Gao, *BioMed Res. Int*. **2015**, *2015*, 125807.
[49] Y. Tanaka, H. Kamohara, K. Kinoshita, J. Kurashige, T. Ishimoto, M. Iwatsuki, M. Watanabe, H. Baba, *Cancer* **2013**, *119*, 1159.
[50] D. D. Taylor, C. Gercel-Taylor, *Gynecol. Oncol*. **2008**, *110*, 13.
[51] A. Becker, B. K. Thakur, J. M. Weiss, H. S. Kim, H. Peinado, D. Lyden, *Cancer Cell* **2016**, *30*, 836.
[52] C. Admyre, S. M. Johansson, K. R. Qazi, J. J. Filén, R. Lahesmaa, M. Norman, E. P. A. Neve, A. Scheynius, S. Gabrielsson, *J. Immunol. Baltim. Md*. **1950**, *179*.
[53] M. Gonzalez-Begne, B. Lu, X. Han, F. K. Hagen, A. R. Hand, J. E. Melvin, J. R. Yates, *J. Proteome Res*. **2009**, *8*, 1304.
[54] A. Poliakov, M. Spilman, T. Dokland, C. L. Amling, J. A. Mobley, *Prostate* **2009**, *69*, 159.
[55] D. S. Choi, J. O. Park, S. C. Jang, Y. J. Yoon, J. W. Jung, D. Y. Choi, J. W. Kim, J. S. Kang, J. Park, D. Hwang, K. H. Lee, S. H. Park, Y. K. Kim, D. M. Desiderio, K. P. Kim, Y. S. Gho, *Proteomics* **2011**, *11*, 2745.
[56] B. D. Lehmann, M. S. Paine, A. M. Brooks, J. A. McCubrey, R. H. Renegar, R. Wang, D. M. Terrian, *Cancer Res*. **2008**, *68*, 7864.
[57] J. Li, C. A. Sherman-Baust, M. Tsai-Turton, R. E. Bristow, R. B. Roden, P. J. Morin, *BMC Cancer* **2009**, *9*, 244.
[58] S. Sharma, F. Zuñiga, G. E. Rice, L. C. Perrin, J. D. Hooper, C. Salomon, *Oncotarget* **2017**, *8*, 104687.
[59] S. A. Melo, L. B. Luecke, C. Kahlert, A. F. Fernandez, S. T. Gammon, J. Kaye, V. S. LeBleu, E. A. Mittendorf, J. Weitz, N. Rahbari, C. Reissfelder, *Nature* **2015**, *523*, 177.
[60] T. Skotland, N. P. Hessvik, K. Sandvig, A. Llorente, *J. Lipid Res*. **2018**, https://doi/org/10.1194/jlr.R084343.
[61] T. Llorente, T. Skotland, D. Sylvanne, T. Kauhanen, A. Rog, I. Orlowski, K. Vattulainen, K. Ekroos, K. Sandvig, *Biochim. Biophys. Acta* **2013**, *1831*, 1302.
[62] T. Skotland, K. Ekroos, D. Kauhanen, H. Simolin, T. Seierstad, V. Berge, K. Sandvig, A. Llorente, *Eur. J. Cancer* **2017**, *70*, 122.
[63] T. Skotland, K. Sandvig, A. Llorente, *Prog. Lipid Res*. **2017**, *66*, 30.
[64] D. W. Greening, R. Xu, H. Ji, B. J. Tauro, R. J. Simpson, *Methods Mol. Biol*. **2015**, *1295*, 179.
[65] P. Li, M. Kaslan, S. H. Lee, J. Yao, Z. Gao, *Theranostics* **2017**, *7*, 789.
[66] M. Y. Konoshenko, E. A. Lekchnov, A. V. Vlassov, P. P. Laktionov, *BioMed Res. Int*. **2018**, *2018*, 8545347.
[67] L. Musante, D. E. Tataruch, H. Holthofer, *Front. Endocrinol*. **2014**, *26*, 149.
[68] P. Fernández-Llama, S. Khositseth, P. A. Gonzales, R. A. Star, T. Pisitkun, M. A. Knepper, *Kidney Int*. **2010**, *77*, 736.
[69] I. Helwa, J. Cai, M. D. Drewry, A. Zimmerman, M. B. Dinkins, M. L. Khaled, M. Seremwe, W. M. Dismuke, E. Bieberich, W. D. Stamer, M. W. Hamrick, *PLoS ONE* **2017**, *12*, e0170628.
[70] K. W. Witwer, E. I. Buzas, L. T. Bemis, A. Bora, C. Lässer, J. Lötvall, E. N. Nolte-'t Hoen, M. G. Piper, S. Sivaraman, J. Skog, C. Théry, M. H. Wauben, F. Hochberg, *J. Extracell Vesicles* **2013**, *2*, 20360.
[71] G. Raposo, H. W. Nijman, W. Stoorvogel, R. Liejendekker, C. V. Harding, C. J. Melief, H. J. Geuze, *J. Exp. Med*. **1996**, *183*, 1161.
[72] B. J. Tauro, D. W. Greening, R. A. Mathias, H. Ji, S. Mathivanan, A. M. Scott, R. J. Simpson, *Methods* **2012**, *56*, 293.
[73] A. Cheruvanky, H. Zhou, T. Pisitkun, J. B. Kopp, M. A. Knepper, P. S. T. Yuen, R. A. Star, *Am. J. Physiol. Renal Physiol*. **2007**, *292*, 1657.
[74] I. M. Rood, J. K. J. Deegens, M. L. Merchant, W. P. M. Tamboer, D. W. Wilkey, M. J. F. Wetzels, J. B. Klein, *Kidney Int*. **2010**, *78*, 810.
[75] Y. Weng, Z. Sui, Y. Shan, Y. Hu, Y. Chen, L. Zhang, Y. Zhang, *Analyst* **2016**, *141*, 4640.
[76] S. Gholizadeh, M. S. Draz, M. Zarghooni, A. Sanati-Nezhad, S. Ghavami, H. Shafiee, M. Akbari, *Biosens. Bioelectron*. **2017**, *91*, 588.
[77] D. W. Greening, R. Xu, H. Ji, B. J. Tauro, R. J. Simpson, *Methods Mol. Biol*. **2015**, *1295*, 179.
[78] M. P. Oksvold, A. Neurauter, K. W. Pedersen, *Methods Mol. Biol*. **2015**, *1218*, 465.
[79] K. W. Hon, N. Abu, N. S. Ab Mutalib, R. Jamal, *Front. Pharmacol*. **2017**, *28*, 583.
[80] G. Pocsfalvi, C. Stanly, A. Vilasi, I. Fiume, R. Tatè, G. Capasso, *World J. Urol*. **2014**, *3*, 66.
[81] D. L. Rupert, C. Lässer, M. Eldh, S. Block, V. P. Zhdanov, J. O. Lotvall, M. Bally, F. Höök, *Anal. Chem*. **2014**, *86*, 5929.
[82] Y. Jiang, M. Shi, Y. Liu, S. Wan, C. Cui, L. Zhang, W. Tan, *Angew. Chem. Int. Ed*. **2017**, *56*, 11916.
[83] Y. Xia, M. Liu, L. Wang, A. Yan, W. He, M. Chen, J. Lan, J. Xu, L. Guan, J. Chen, *Biosens. Bioelectron*. **2017**, *92*, 8.
[84] H. Im, H. Shao, Y. I. Park, V. M. Peterson, C. M. Castro, R. Weissleder, H. Lee, *Nat. Biotechnol*. **2014**, *32*, 490.
[85] K. S. Yang, H. Im, S. Hong, I. Pergolini, A. F. Del Castillo, R. Wang, S. Clardy, C. H. Huang, C. Pille, S. Ferrone, R. Yang, C. M. Castro, H. Lee, C. F. Del Castillo, R. Weissleder, *Sci. Transl. Med*. **2017**, *9*, 3226.
[86] S. Jeong, J. Park, D. Pathania, C. M. Castro, R. Weissleder, H. Lee, *ACS Nano* **2016**, *10*, 1802.
[87] J. Park, H. Y. Lin, J. P. Assaker, S. Jeong, C. H. Huang, A. Kurdi, K. Lee, K. Fraser, C. Min, S. Eskandari, S. Routray, *ACS Nano* **2017**, *11*, 11041.
[88] Y. M. Wang, J. W. Liu, G. B. Adkins, W. Shen, M. P. Trinh, L. Y. Duan, J. H. Jiang, W. Zhong, *Anal. Chem*. **2017**, *89*, 12327.
[89] L. J. Van't Veer, R. Bernards, *Nature* **2008**, *452*, 564.
[90] C. P. Carden, D. Sarker, S. Postel-Vinay, T. A. Yap, G. Attard, U. Banerji, M. D. Garrett, G. V. Thomas, P. Workman, S. B. Kaye, J. S. de Bono, *Drug Discov. Today* **2010**, *5*, 88.

[91] L. Gorgannezhad, M. Umer, M. N. Islam, N. T. Nguyen, M. J. Shiddiky, *Lab Chip* **2018**, *18*, 1174.
[92] L. Raimondi, A. De Luca, V. Costa, N. Amodio, V. Carina, D. Bellavia, P. Tassone, S. Pagani, M. Fini, R. Alessandro, G. Giavaresi, *Oncotarget* **2017**, *8*, 100831.
[93] A. S. Azmi, B. Bao, F. H. Sarkar, *Cancer Metastasis Rev.* **2013**, *32*, 623.
[94] T. Li, R. Zhang, H. Chen, Z. Huang, X. Ye, H. Wang, J. Kong, *Chem. Sci*. **2018**, *9*, 5372.
[95] K. S. Sheinerman, S. R. Umansky, *Front. Cell Neurosci*. **2013**, *10*, 150.
[96] K. S. Park, *Biosens. Bioelectron*. **2018**, *102*, 179.
[97] D. S. Wilson, J. W. Szostak, *Annu. Rev. Biochem*. **1999**, *68*, 611.
[98] T. H. Ku, T. Zhang, H. Luo, T. M. Yen, P. W. Chen, Y. Han, Y. H. Lo, *Sensors* **2015**, *15*, 16281.
[99] W. Guo, Y. Gao, N. Li, F. Shao, C. Wang, P. Wang, Z. Yang, R. Li, J. He, *Oncol. Rep*. **2017**, *38*, 665.
[100] S. Gholizadeh, M. S. Draz, M. Zarghooni, A. Sanati-Nezhad, S. Ghavami, H. Shafiee, M. Akbari, *Biosens. Bioelectron*. **2017**, *91*, 588.
[101] H. Xu, C. Liao, P. Zuo, Z. Liu, B. C. Ye, *Anal. Chem*. **2018**, https://doi.org/10.1021/acs.analchem.8b03272.
[102] K. L. Schey, J. M. Luther, K. L. Rose, *Methods* **2015**, *87*, 75.
[103] X. Zhu, X. Wang, S. Wei, Y. Chen, Y. Chen, X. Fan, S. Han, G. Wu, *FEBS J*. **2017**, *284*, 2170.
[104] J. T. Yao, S. H. Zhao, Q. P. Liu, M. Q. Lv, D. X. Zhou, Z. J. Liao, K. J. Nan, *Pathol. Res. Pract*. **2017**, *213*, 453.
[105] R. J. Lobb, R. van Amerongen, A. Wiegmans, S. Ham, J. E. Larsen, A. Moller, *Int. J. Cancer* **2017**, *141*, 614.
[106] R. Zhang, Y. Xia, Z. Wang, J. Zheng, Y. Chen, X. Li, Y. Wang, H. Ming, *Biochem. Biophys. Res. Commun*. **2017**, *490*, 406.
[107] H. Dejima, I. H. Inuma, R. Kanaoka, N. Matsutani, M. Kawamura, *Oncol. Lett*. **2017**, *13*, 1256.
[108] B. Schmidt, G. Rehbein, M. Fleischhacker, *Adv. Exp. Med. Biol*. **2016**, *924*, 33.
[109] J. Zheng, X. Liu, Y. Xue, W. Gong, J. Ma, Z. Xi, Z. Que, Y. Liu, *J. Hematol. Oncol*. **2017**, *10*, 52.
[110] J. Skog, T. Wurdinger, S. van Rijn, D. H. Meijer, L. Gainche, M. Sena-Esteves, W. T. CurryJr, B. S. Carter, A. M. Krichevsky, O. X. Breakefield, *Nat. Cell Biol*. **2008**, *10*, 1470.
[111] K. Zhang, X. Sun, X. Zhou, L. Han, L. Chen, Z. Shi, A. Zhang, M. Ye, Q. Wang, C. Liu, J. Wei, Y. Ren, J. Yang, J. Zhang, P. Pu, M. Li, C. Kang, *Oncotarget* **2015**, *6*, 537.
[112] H. Shao, J. Chung, L. Balaj, A. Charest, D. D. Bigner, B. S. Carter, F. H. Hochberg, X. O. Breakefield, R. Weissleder, H. Lee, *Nat. Med*. **2012**, *18*, 1835.
[113] S. Dijkstra, I. L. Birker, F. P. Smit, G. H. Leyten, T. M. de Reijke, I. M. van Oort, P. F. Mulders, S. A. Jannink, J. A. Schalken, *J. Urol*. **2014**, *191*, 1132.
[114] M. Isin, E. Uysaler, E. Ozgur, H. Köseoğlu, Ö. Şanlı, Ö. B. Yücel, U. Gezer, N. Dalay, *Front. Genet*. **2015**, *6*, 168.
[115] Z. Kong, X. Wan, Y. Zhang, P. Zhang, Y. Zhang, X. Zhang, X. Qi, H. Wu, J. Huang, Y. Li, *Biochem. Biophys. Res. Commun*. **2017**, *493*, 1217.
[116] J. A. March-Villalba, J. M. Martínez-Jabaloyas, M. J. Herrero, J. Santamaria, S. F. Aliño, F. Dasí, *PLoS ONE* **2012**, *7*, e43470.
[117] A. Chronopoulos, T. J. Lieberthal, A. E. del Río Hernández, *Converg. Sci. Phys. Oncol*. **2017**, *3*, 013005.
[118] M. Logozzi, D. F. Angelini, E. Iessi, D. Mizzoni, R. Di Raimo, C. Federici, L. Lugini, G. Borsellino, A. Gentilucci, F. Pierella, V. Marzio, *Cancer Lett*. **2017**, *403*, 318.
[119] K. Kawakami, Y. Fujita, Y. Matsuda, T. Arai, K. Horie, K. Kameyama, T. Kato, K. Masunaga, Y. Kasuya, M. Tanaka, K. Mizutani, *BMC Cancer* **2017**, *17*, 316.
[120] A. Gamez-Valero, S. I. Lozano-Ramos, I. Bancu, R. Lauzurica-Valdemoros, F. E. Borras, *Front. Immunol*. **2015**, *6*, 6.
[121] L. Dong, W. R. Lin, P Qi, M. D. Xu, X. Wu, S. Ni, D. Huang, W. W. Weng, C. Tan, W. Sheng, X. Zhou, X. Du, *Cancer Epidemiol. Biomark Prev*. **2016**, *25*, 1158.
[122] Y. Li, Q. Zheng, C. Bao, S. Li, W. Guo, J. Zhao, D. Chen, J. Gu, X. He, S. Huang, *Cell Res*. **2015**, *25*, 981.
[123] M. Zhu, Y. Xu, Y. Chen, F. Yan, *Biomed. Pharmacother*. **2017**, *88*, 138.
[124] X. L. Zhang, L. L. Xu, F. Wang, *Cell Biol. Int*. **2017**, *41*, 1056.
[125] T. Liu, X. Zhang, S. Gao, F. Jing, Y. Yang, L. Du, G. Zheng, P. Li, C. Li, C Wang, *Oncotarget* **2016**, *7*, 85551.
[126] L. Pan, W. Liang, M. Fu, Z. H. Huang, X. Li, W. Zhang, P. Zhang, H. Qian, P. C. Jiang, W. R. Xu, X. Zhang, *J. Cancer Res. Clin. Oncol*. **2017**, *143*, 991.
[127] Y. Dou, D. J. Cha, J. L. Franklin, J. N. Higginbotham, D. K. Jeppesen, A. M. Weaver, N. Prasad, S. Levy, R. J. Coffey, J. G. Patton, B. Zhang, *Sci. Rep*. **2016**, *6*, 37982.
[128] T. Matsumura, K. Sugimachi, H. Iinuma, Y. Takahashi, J. Kurashige, G. Sawada, M. Ueda, R. Uchi, H. Ueo, Y. Takano, Y. Shinden, *Br. J. Cancer* **2015**, *113*, 275.
[129] H. Ogata-Kawata, M. Izumiya, D. Kurioka, Y. Honma, Y. Yamada, K. Furuta, T. Gunji, H. Ohta, H. Okamoto, H. Sonoda, M. Watanabe, *PLoS ONE* **2014**, *9*, e92921.
[130] Y. Teng, Y. Ren, X. Hu, J. Mu, A. Samykutty, X. Zhuang, Z. Deng, A. Kumar, L. Zhang, M. L. Merchant, J. Yan, *Nat. Commun*. **2017**, *8*, 14448.
[131] R. Uratani, Y. Toiyama, T. Kitajima, M. Kawamura, J. Hiro, M. Kobayashi, K. Tanaka, Y. Inoue, Y. Mohri, T. Mori, T. Kato, *PLoS ONE* **2016**, *11*, e0160722.
[132] K. Allenson, J. Castillo, F. A. San Lucas, G. Scelo, D. U. Kim, V. Bernard, G. Davis, T. Kumar, M. Katz, M. J. Overman, L. Foretova, *Ann. Oncol*. **2017**, *28*, 741.
[133] U. Warnecke-Eberz, S. H. Chon, A. H. Hölscher, U. Drebber, E. Bollschweiler, *Tumour Biol*. **2015**, *36*, 4643.
[134] Z. Li, P. Jiang, J. Li, M. Peng, X. Zhao, X. Zhang, K. Chen, Y. Zhang, H. Liu, L. Gan, H. Bi, *Oncogene* **2018**, *37*, 3822.
[135] J. Li, Z. Li, P. Jiang, M. Peng, X. Zhang, K. Chen, H. Liu, H. Bi, X. Liu, X. Li, *J. Exp. Clin. Cancer Res*. **2018**, *37*, 177.
[136] J. F. Xu, Y. P. Wang, S. J. Zhang, Y. Chen, H. F. Gu, X. F. Dou, B. Xia, Q. Bi, S. W. Fan, *Oncotarget* **2017**, *8*, 75968.
[137] Z. Kun-Peng, M. Xiao-Long, Z. Lei, Z. Chun-Lin, H. Jian-Ping, Z. Tai-Cheng, *Epigenomics* **2018**, *10*, 1327.
[138] B. N. Hannafon, Y. D. Trigoso, C. L. Calloway, Y. D. Zhao, D. H. Lum, A. L. Welm, Z. J. Zhao, K. E. Blick, W. C. Dooley, W. Q. Ding, *Breast Cancer Res*. **2016**, *18*, 90.
[139] C. Liu, C. Eng, J. Shen, Y. Lu, T. Yoko, A. Mehdizadeh, G. J. Chang, M. A. Rodriguez-Bigas, Y. Li, P. Chang, *Oncotarget* **2016**, *7*, 76250.
[140] P. Jenjaroenpun, Y. Kremenska, V. M. Nair, M. Kremenskoy, B. Joseph, I. V. Kurochkin, *PeerJ* **2013**, *1*, e201.
[141] C. G. Xu, M. F. Yang, Y. Q. Ren, C. H. Wu, L. Q. Wang, *Eur. Rev. Med. Pharmacol. Sci*. **2016**, *20*, 4362.
[142] Y. Tanaka, H. Kamohara, K. Kinoshita, J. Kurashige, T. Ishimoto, M. Iwatsuki, *Cancer* **2013**, *119*, 1159.
[143] J. Liu, H. Sun, X. Wang, Q. Yu, S. Li, X. Yu, W. Gong, Int J Mol, *Science* **2014**, *15*, 758.
[144] W. Duan, L. Du, X. Jiang, R. Wang, S. Yan, Y. Xie, K. Yan, Q. Wang, L. Wang, X. Zhang, H. Pan, Y. Yang, C. Wang, *Oncotarget* **2016**, *29*, 78850.
[145] C. Berrondo, J. Flax, V. Kucherov, A. Siebert, T. Osinski, A. Rosenberg, C. Fucile, S. Richheimer, C. J. Beckham, *PLoS ONE* **2016**, *11*, 0147236.
[146] Y. Yoshioka, N. Kosaka, Y. Konishi, H. Ohta, H. Okamoto, H. Sonoda, R. Nonaka, H. Yamamoto, H. Ishii, M. Mori, K. Furuta, *Nat. Commun*. **2014**, *5*, 3591.

[147] J. Li, Y. Chen, X. Guo, L. Zhou, Z. Jia, Z. Peng, Y. Tang, W. Liu, B. Zhu, L. Wang, C. Ren, *J. Cell Mol. Med*. **2017**, *21*, 838.
[148] S. Yokoyama, A. Takeuchi, S. Yamaguchi, Y. Mitani, T. Watanabe, K. Matsuda, T. Hotta, J. E. Shively, H. Yamaue, *PLoS ONE* **2017**, *12*, e0183337.
[149] P. G. Moon, J. E. Lee, Y. E. Cho, S. J. Lee, J. H. Jung, Y. S. Chae, H. I. Bae, Y. B. Kim, I. S. Kim, H. Park, M. C. Baek, *Clin. Cancer Res*. **2015**, *24*, clincanres-0654.
[150] P. G. Moon, J. E. Lee, Y. E. Cho, S. J. Lee, Y. S. Chae, J. H. Jung, I. S. Kim, H. Y. Park, M. C. Baek, *Oncotarget* **2016**, *7*, 40189.
[151] S. Khan, H. F. Bennit, D. Turay, M. Perez, S. Mirshahidi, Y. Yuan, N. R. Wall, *BMC Cancer* **2014**, *14*, 176.
[152] I. H. Chen, L. Xue, C. C. Hsu, J. S. Paez, L. Pan, H. Andaluz, M. K. Wendt, A. B. Iliuk, J. K. Zhu, W. A. Tao, *Proc. Natl. Acad. Sci*. **2017**, *114*, 3175.
[153] S. Fang, H. Tian, X. Li, D. Jin, X. Li, J. Kong, C. Yang, X. Yang, Y. Lu, Y. Luo, B. Lin, *PLoS ONE* **2017**, *2*, e0175050.
[154] K. R. Jakobsen, B. S. Paulsen, R. Bæk, K. Varming, B. S. Sorensen, M. M. Jørgensen, *J Extracell Vesicles*. **2015**, *4*, 26659.
[155] Y. Li, Y. Zhang, F. Qiu, Z. Qiu, *Electrophoresis* **2011**, *32*, 1976.
[156] T. Yamashita, H. Kamada, S. Kanasaki, Y. Maeda, K. Nagano, Y. Abe, M. Inoue, Y. Yoshioka, Y. Tsutsumi, S. Katayama, S. Tsunoda, *Int. J. Pharm. Sci*. **2013**, *68*, 969.
[157] P. Reclusa, S. Taverna, M. Pucci, E. Durendez, S. Calabuig, P. Manca, M. J. Serrano, L. Sober, P. Pauwels, A. Russo, *J. Thorac Dis*. **2017**, *9*, S1373.
[158] L. Z. Wang, R. A. Soo, W. L. Thuya, T. T. Wang, T. Guo, J. A. Lau, F. C. Wong, A. L. Wong, S. C. Lee, S. K. Sze, B. C. Goh, *J. Clin. Oncol*. **2014**, *32*, e22162.
[159] M. Herreros-Villanueva, L. Bujanda, *Ann. Transl. Med*. **2016**, *7*, 134.
[160] B. Costa-Silva, N. M. Aiello, A. J. Ocean, S. Singh, H. Zhang, B. K. Thakur, A. Becker, A. Hoshino, M. T. Mark, H. Molina, J. Xiang, *Nat. Cell Biol*. **2015**, *17*, 816.
[161] A. Chronopoulos, T. J. Lieberthal, A. E. del Río Hernández, *Converg. Sci. Phys. Oncol*. **2017**, *3*, 013005.
[162] C. Keryer-Bibens, C. Pioche-Durieu, C. Villemant, S. Souquère, N. Nishi, M. Hirashima, J. Middeldorp, P. Busson, *BMC Cancer* **2006**, *6*, 283.
[163] M. Szajnik, M. Derbis, M. Lach, P. Patalas, M. Michalak, H. Drzewiecka, D. Szpurek, A. Nowakowski, M. Spaczynski, W. Baranowski, T. L. Whiteside, *Gynecol. Obstetr*. **2013**, *29*, 003.
[164] Z. Zhao, Y. Yang, Y. Zeng, M. He, *Lab Chip* **2016**, *16*, 489.
[165] J. Li, C. A. Sherman-Baust, M. Tsai-Turton, R. E. Bristow, R. B. Roden, P. J. Morin, *BMC Cancer* **2009**, *9*, 244.
[166] B. Liang, P. Peng, S. Chen, L. Li, M. Zhang, D. Cao, J. Yang, H. Li, T. Gui, X. Li, K. Shen, *J. Proteom*. **2013**, *27*, 171.
[167] V. O. Shender, M. S. Pavlyukov, R. H. Ziganshin, G. P. Arapidi, S. I. Kovalchuk, N. A. Anikanov, I. A. Altukhov, D. G. Alexeev, I. O. Butenko, A. L. Shavarda, E. Khomyakova, *Mol. Cell Proteom*. **2014**, *12*, 3558.
[168] S. Runz, S. Keller, C. Rupp, A. Stoeck, Y. Issa, D. Koensgen, A. Mustea, J. Sehouli, G. Kristiansen, P. Altevogt, *Gynecol. Oncol*. **2007**, *07*, 563.
[169] W. Li, C. Li, T. Zhou, X. Liu, X. Liu, X. Li, D. Chen, *Mol. Cancer* **2017**, *16*, 145.
[170] C. L. Chen, Y. F. Lai, P. Tang, K. Y. Chien, J. S. Yu, C. H. Tsai, H. W. Chen, C. C. Wu, T. Chung, C. W. Hsu, C. D. Chen, *J. Proteome Res*. **2012**, *11*, 5611.
[171] D. M. Smalley, N. E. Sheman, K. Nelson, D. Theodorescu, *J. Proteome Res*. **2008**, *7*.
[172] M. Logozzi, A. De Milito, L. Lugini, M. Borghi, L. Calabro, M. Spada, M. Perdicchio, M. L. Marino, C. Federici, E. Iessi, D. Brambilla, *PLoS ONE* **2009**, *4*, e5219.
[173] A. L. Revenfeld, R. Bæk, M. H. Nielsen, A. Stensballe, K. Varming, M. Jørgensen, *Clin. Ther*. **2014**, *36*, 830.
[174] F. Raimondo, L. Morosi, S. Corbetta, C. Chinello, P. Brambilla, P. Della Mina, A. Villa, G. Albo, C. Battaglia, S. Bosari, *Mol. BioSys*. **2013**, *9*, 1220.
[175] J. Baran, M. Baj-Krzyworzeka, K. Weglarczyk, R. Szatanek, M. Zembala, J. Barbasz, A. Czupryna, A. Szczepanik, M. Zembala, *Cancer Immunol. Immunother*. **2010**, *59*, 841.
[176] I. Parolini, C. Federici, C. L. Raggi, Lugini, S. Palleschi, A. De Milito, C. Coscia, E. Iessi, M. A. Logozzi, A. Molinari, M. Colone, *J. Biol. Chem*. **2009**, *284*, 34211.
[177] C. Beyer, D. S. Pisetsky, *Nat. Rev. Rheumatol*. **2010**, *6*, 21.
[178] A. N. Boing, E. van der Pol, A. E. Grootemaat, F. A. W. Coumans, A. Sturk, R. Nieuwland, *J. Extracell Vesicles*. **2014**, *3*, 25922.
[179] R. Grant, E. Ansa-Addo, D. Stratton, S. Antwi-Baffour, S. Jorfi, S. Kholia, L. Krige, S. Lange, J. Inal, *J. Immunol. Methods* **2011**, *371*, 143.
[180] D. W. Greening, R. Xu, H. Ji, B. J. Tauro, R. J. Simpson, *Methods Mol. Biol*. **2015**, *1295*, 179.