Research Advances on Exosomes as Tumor Biomarkers: Postprint

Authors: Liu Yan, Peng Dai, Zhu Yunfeng

Date: 2019-01-17T00:00:00+00:00

Abstract

Exosomes are intracellular vesicle-like biological nanoscale membrane structures with diameters ranging from 40-100 nm that can be secreted and released by various cell types. They possess numerous functions, including intercellular transport and delivery of proteins, mRNA, miRNA, and lipids, antigen presentation, and potentially oncogenic capabilities. Exosomes secreted by tumor cells play important roles in physiological and pathological processes such as tumor initiation, progression, and metastasis. Currently, the search for specific biomarkers from tumor exosomes has become a key focus for cancer researchers, holding significant importance for early tumor diagnosis, therapeutic efficacy evaluation, and prognostic analysis. This article reviews recent research progress on exosomes in tumor research and diagnosis.

Full Text

Research Progress of Exosomes as Tumor Markers

LIU Yan[1],[2], **DAI Peng**[2], **ZHU Yun-feng**[1],

[1]College of Life Sciences and Bioengineering, Beijing Jiaotong University, Beijing 100044, China
[2]Henan Province OriginBio Biotechnology Co., Ltd., Zhengzhou 450000, China

Abstract

Exosomes are naturally occurring biological nanomembranous vesicles (40–100 nm) of endocytic origin that are released into the extracellular space from diverse cell types. They have pleiotropic functions such as antigen presentation and intercellular transfer of proteins, RNAs, and lipids. Exosomes secreted by tumor cells play important roles in the physiological and pathological processes involved in tumor initiation, progression, and metastasis. Currently, the identification of specific markers from tumor exosomes has become a major focus

of cancer research, given their significant potential for early diagnosis, efficacy evaluation, and prognostic analysis. This review summarizes recent advances in exosome research and their applications in tumor diagnosis.

Keywords: tumor; exosome; molecular marker

In the early 1980s, Johnstone et al. [1] isolated a vesicle-like structure approximately 50 nm in size containing transferrin receptors from cell culture media during the maturation of reticulocytes into erythrocytes, which they named "exosomes." It is now recognized that exosomes are polymorphic vesicular structures with diameters ranging from 40–100 nm, secreted by various cell types. Under both physiological and pathological conditions, numerous cells can secrete and release exosomes, including tumor cells [2]. Exosomes contain a variety of receptor proteins and genetic materials such as nucleic acids, serving as important mediators of intercellular signal transmission. These nucleic acids and proteins can reflect specific physiological and pathological states, and exosomes released from tumor cells play crucial roles in tumor occurrence, development, and metastasis.

1.1 Main Components of Exosomes

Exosomes originate from late endosomes (also known as multivesicular bodies, MVBs), which form when endosomal membranes invaginate to create structures containing multiple small vesicles. The specific composition of exosomes varies depending on their cell of origin.

Proteins constitute one of the main components of exosomes and can be broadly classified into two categories. The first category comprises structural proteins that are universally present in exosomes, including cytoskeletal components such as actin and microfilament-associated proteins, as well as membrane proteins, lysosomal proteins (Lamp2b), and heat shock proteins (hsp70, hsp90) [4,5]. The second category consists of specific proteins that exist only in exosomes derived from particular cell types, conferring relative specificity. For example, exosomes from dendritic cells contain various surface-associated proteins (such as Alix, TSG101) and tetraspanins (such as CD63, CD9) [6], whereas exosomes derived from nasopharyngeal carcinoma carry Epstein-Barr virus protein LMP1 (Latent Membrane Protein 1), which can promote immune evasion by cancer cells [7]. Additionally, tumor antigens and immunosuppressive proteins such as FasL, TRAIL, and TGF- can be detected in exosomes from other tumor cells, and their overexpression suggests the potential of exosomal proteins as diagnostic and therapeutic evaluation markers for tumors and related diseases.

Furthermore, various nucleic acid components have been detected within exosomes, including mRNA, miRNA, long non-coding RNAs (lncRNAs), and circRNAs [8]. Studies have shown that exosomal RNA levels differ among cells, with variations in both the types and expression quantities of RNA across differ-

ent tumor types. Moreover, the expression levels and types of exosomal nucleic acids change under different physiological and pathological conditions [9–11]. For instance, miR-182 and miR-183 are highly overexpressed in ductal carcinoma in situ of the breast compared to normal tissue, promoting breast cancer initiation and metastasis [12]. Enrichment of circRNAs has also been detected in exosomes from liver cancer cells [13]. Currently, miRNAs from tumor-derived exosomes have become a major focus of research interest, as they are ubiquitous and highly abundant, playing important regulatory roles in tumor initiation and progression [14,15].

1.2 Formation and Cargo Sorting of Exosomes

As early endosomes mature into late endosomes, they engulf sorted proteins and nucleic acids, forming multiple intraluminal vesicles (ILVs) through budding—the precursors of exosomes. Late endosomes containing multiple ILVs are termed multivesicular bodies (MVBs). Some MVBs fuse with lysosomes for degradation of their contents, while others are transported to the cell periphery by molecular motors and secreted into the extracellular space, ultimately forming exosomes [16]. Current research suggests two main mechanisms for exosomal cargo sorting: ESCRT-dependent and ESCRT-independent pathways.

The endosomal sorting complex required for transport (ESCRT) is a shaping protein complex located on the cytoplasmic side of endosomes that can recognize ubiquitinated membrane proteins and sort specific nucleic acids and proteins into ILVs to form exosome precursors. ESCRT comprises five core complexes: four subcomplexes (ESCRT-0, I, II, III) and the accessory protein Vps4-Vta1 [17]. The components and functions of these four ESCRT complexes differ. ESCRT-0 recognizes and enriches ubiquitized cargo proteins on endosomal membranes. ESCRT-I and ESCRT-II work synergistically to package sorted specific proteins and nucleic acids into MVBs and induce MVB budding to form initial buds (exosomes). ESCRT-III then severs these buds, releasing the exosomes [18]. Finally, Vps4 disassembles ESCRT components for recycling.

Additionally, cells can generate ILVs and MVBs independently of ESCRT through the assistance of lipids, ceramide, or heat shock proteins. Stuffers et al. [19] depleted all four ESCRT subcomplexes to maximally inhibit ESCRT-dependent pathways, yet still observed release of CD63-positive exosomes, suggesting that exosomes can also form via ESCRT-independent mechanisms. The first ESCRT-independent mechanism involves neutral sphingomyelinase (nSMase) generating ceramide; inhibition of nSMase can reduce exosome production [20]. Moreover, studies have demonstrated that members of the tetraspanin family, including CD63, CD81, and CD9, participate in ESCRT-independent cargo sorting into vesicles [21,22].

1.3 Physiological Functions of Exosomes

Exosomes are widely present in bodily fluids and tissues. Early studies considered exosomes to function as "garbage bags" for cellular waste removal. However, recent research indicates that exosomes are closely involved in various biological processes.

First, exosomes participate in intercellular signal exchange and transmission through autocrine, paracrine, and endocrine mechanisms. This occurs primarily in two ways. On one hand, exosomes can release active cargo that contacts and fuses with target cells, regulating intracellular physiological activities. For example, exosomes from certain cells contain proteins involved in signaling pathways such as -catenin and WNT5B, as well as mRNA and miRNA, which can be transported to target cells to modulate gene expression [23]. On the other hand, exosomes can directly transmit information through surface signaling molecules. Wang et al. [24] found that under conditions of high reactive oxygen species or oxidative stress in neurons, glial cell-secreted exosomes contain synaptophysin that promotes neuronal survival and axonal growth.

Second, exosomes contain numerous immune-related molecules and participate in immune regulation. Vlassov et al. [25] reported that in pregnant women' s serum, placenta-derived exosomes containing FasL can inhibit CD3 and JAK3 expression in T cell signaling pathways, potentially representing one mechanism by which the placenta promotes immune privilege. Clotilde et al. [26] also found that dendritic cell-derived exosomes contain MHC I and CD86, which can induce CD8+ T cells to kill tumor cells, demonstrating certain anti-tumor effects. Furthermore, exosomes are involved in antigen presentation. Hao et al. [27] discovered that exosomes from dendritic cells pulsed with ovalbumin, when taken up by mature dendritic cells, promote antigen cross-presentation and CD8+ T cell proliferation, thereby more effectively inducing ovalbumin-specific CTL responses and anti-tumor immunity.

Finally, exosomes serve as cargo carriers. Based on their structural characteristics and biological functions, using exosomes as drug delivery vehicles and therapeutic systems for malignant tumors has become a hot topic in recent research. Alvarez et al. [28] transfected dendritic cells with an expression plasmid for rabies virus glycoprotein-lysosome-associated membrane protein (RVG-Lamp2b), enabling dendritic cells to secrete exosomes displaying RVG peptides. They then loaded BACE1 siRNA and GAPDH siRNA plasmids into these RVG-peptide-conjugated exosomes using electroporation, ultimately obtaining targeted RVG-Exos complexes. Additionally, Qi and colleagues modified superparamagnetic Fe3O4 nanoparticles with transferrin on their surface, which bound to specific transferrin receptors on exosomes from immature erythrocytes to create superparamagnetic nanoparticle/exosome complexes (SMNC-Exos) for targeted delivery of doxorubicin in anti-tumor therapy [29].

2 Exosomes and Potential Tumor Markers

Exosomes play important roles in tumor initiation, progression, and metastasis. Tumor cell-derived exosomes carry proteins and nucleic acids characteristic of their parent cells, providing novel targets and strategies for early tumor diagnosis and treatment.

2.1 Lung Cancer Lung cancer is a highly lethal disease with the highest incidence and mortality rates worldwide. Although significant progress has been made in lung cancer research in recent years, the five-year survival rate remains low due to inadequate early diagnosis and high recurrence rates after treatment [30]. In a study by Ueda et al. [31], exosomes from serum of 46 non-small-cell lung carcinoma (NSCLC) patients were analyzed using anti-CD9-MISA combined with LC-MS/MS. The results showed that CD91 had a diagnostic sensitivity of 72%, specificity of 60%, and an area under the ROC curve (AUC) of 89%, suggesting its potential as a biomarker for NSCLC diagnosis. Additionally, expression levels of let-7f, miR-20b, miR-30e-3p, miR-25, and miR-223 in exosomes from lung cancer patient fluids differed significantly from those in healthy individuals [32].

2.2 Pancreatic Cancer Pancreatic cancer, known as the "king of cancers," has high malignancy and mortality rates with the poorest prognosis. Due to its subtle early symptoms and poor specificity of current clinical tumor markers, most cases are diagnosed at advanced stages. Recent research suggests that exosome detection may solve this problem. Melo' s team analyzed serum from pancreatic cancer patients and found that glypican-1 (GPC1) was highly abundant and highly specific on the surface of serum exosomes from both early- and late-stage patients, indicating that GPC1 could serve as a marker for early pancreatic cancer diagnosis [33]. Furthermore, Que et al. [34] reported that expression levels of miR-17-5p and miR-21 were significantly elevated in serum exosomes from pancreatic cancer patients, and high miR-17-5p expression could serve as an indicator for metastasis and staging.

2.3 Colorectal Cancer Colorectal cancer is one of the most common malignant tumors, ranking second among digestive tract cancers and third worldwide in incidence and mortality. Current endoscopic diagnosis is inefficient and costly, with high recurrence rates, making the search for early diagnostic and prognostic markers critically important. Tauro et al. [35] found that EpCAM, CLDN7, and CD44 expression levels were upregulated on the surface of serum exosomes from colorectal cancer patients, regulating tumor development and progression. Hiroko et al. [36] also discovered that even in early-stage primary colorectal cancer patients, expression levels of seven miRNAs (let-7a, miR-1229, etc.) were significantly elevated in serum exosomes and decreased markedly after tumor resection, suggesting these miRNAs could serve as potential non-invasive biomarkers for early colorectal cancer diagnosis. Additionally, Hu et al. [37] identified six lncRNAs that were significantly upregulated in plasma

exosomes from colorectal cancer patients, which could serve as biomarkers for early diagnosis.

2.5 Ovarian Cancer As one of the common gynecological malignant tumors, ovarian cancer causes approximately 130,000 deaths annually, with incidence rates second only to cervical and endometrial cancers. The low early diagnosis rate and high recurrence rate necessitate the discovery of novel specific markers to improve diagnostic accuracy. Szajnik et al. [38] detected differences in plasma exosome protein content between malignant ovarian cancer patients and benign tumor patients, finding significantly elevated expression of MAGE 3/6 and TGF- 1 (transforming growth factor beta 1) in ovarian cancer patient plasma exosomes, suggesting these could serve as protein markers for early diagnosis and prognostic evaluation. Ying et al. [39] also reported that ovarian cancer-derived exosomes are enriched in miR-222-3p, which can be transferred to macrophages to promote tumor-associated macrophage polarization, making it a potential biomarker for ovarian cancer.

2.6 Prostate Cancer Prostate cancer is the most common malignant tumor in men and ranks among the top causes of cancer mortality worldwide. Current clinical diagnostic markers have low specificity, and prostate biopsy is painful for patients. Therefore, identifying molecular markers with high specificity, diagnostic accuracy, and minimal invasiveness has become a research priority. Li et al. [40] analyzed exosomes from serum samples of prostate cancer patients, benign prostatic hyperplasia patients, and healthy volunteers, comparing miR-141 expression levels in whole serum versus serum exosomes and their correlation with clinicopathological features. They found significantly elevated miR-141 levels, with ROC curve analysis showing an AUC of 0.8694, sensitivity of 80%, and specificity of 87.1%, suggesting serum exosomal miR-141 as a potential biomarker for metastatic prostate cancer diagnosis. Additionally, TM256 protein in urine exosomes from prostate cancer patients showed very high diagnostic sensitivity and specificity [41].

2.7 Bladder Cancer Bladder cancer, occurring in the bladder mucosal lining, is a common urinary system tumor with increasing incidence rates in China. Since the bladder stores urine, identifying early diagnostic markers from urinary exosomes offers significant advantages. Beckham et al. [42] reported that urinary exosomes from high-grade bladder cancer patients contain the bioactive molecule EDIL-3, which has diagnostic marker value. Long et al. [43] analyzed 236 miRNAs in exosomes from bladder transitional cell carcinoma patients and controls using PCR arrays, finding differential expression of seven miRNAs with 88% sensitivity and 78% specificity for bladder transitional cell carcinoma. Furthermore, recent studies have found that lncRNA PTENP1 expression is significantly downregulated in exosomes from bladder cancer patient tissues and plasma [44], suggesting both exosomal miRNA and lncRNA PTENP1 could serve as novel markers for bladder cancer diagnosis and treatment.

2.8 Breast Cancer Breast cancer is a major threat to women' s health, with early detection and diagnosis being key to improving treatment outcomes. Halvaei et al. [45] found significantly elevated expression of miRNAs (including miR-21, miR-195, miR-484, and miR-1246) in serum exosomes from breast cancer patients. Other studies have indicated that serum exosomal circRNA expression levels can distinguish breast cancer patients from healthy controls [46], demonstrating that exosomal RNAs can serve as diagnostic biomarkers for breast cancer.

2.9 Other Tumors Beyond the aforementioned cancers, significant changes in exosomal proteins and nucleic acids have been detected in other common human tumors. For example, RNA-seq analysis of exosomal circRNA expression in serum from endometrial cancer patients and healthy volunteers revealed that the number of upregulated circRNAs was significantly higher than downregulated circRNAs in endometrial cancer patient exosomes [47]. In liver cancer patients, serum exosomal miR-21 expression was significantly higher than in chronic hepatitis B patients or healthy volunteers and correlated with liver cancer staging [48]. Additionally, Fujiwara et al. [49] found that miR-17-5p and miR-25-3p were significantly elevated in serum from osteosarcoma patients, with serum exosomal miR-25-3p levels correlating with patient prognosis. Renal cell carcinoma-derived exosomes contain large amounts of the renal cancer-specific antigen G250 [50]. These findings indicate that the close relationship between exosomal proteins and nucleic acids with tumors endows them with potential as biomarkers for tumor diagnosis and treatment.

Exosomes are widely present in various cell types and play important roles in the transport of cellular materials and genetic information. On one hand, the endogenous nanomembrane structural characteristics of exosomes offer significant advantages for drug loading and can be engineered for specific targeting of disease sites to deliver drugs and small molecules, providing a new research direction for precision tumor therapy. On the other hand, exosomes carry nucleic acids and proteins characteristic of their parent cells under both physiological and pathological conditions. Therefore, investigating exosomes as biomarkers for early tumor diagnosis and prognostic evaluation holds important clinical significance for improving survival rates and outcomes in cancer patients.

References

[1] Pan BT, Johnstone RM. Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor[J]. Cell, 1983, 33(3):967-978.

[2] Zheng H, Zhan Y, Liu S, et al. The roles of tumor-derived exosomes in non-small cell lung cancer and their clinical implications[J]. J Exp Clin Cancer Res, 2018, 37(1):226.

[3] Hartwig S, De Filippo E, Göddeke S, et al. Exosomal proteins constitute an essential part of the human adipose tissue secretome[J]. Biochim Biophys Acta Proteins Proteom, 2018, pii: S1570-9639(18)30207-3.

[4] Graner MW, Cumming RI, Bigner DD. The heat shock response and chaperones/heat shock proteins in brain tumors: surface expression, release, and possible immune consequences[J]. J Neurosci, 2007, 27(42):11214-11227.

[5] Colombo M, Moita C, Van NG, et al. Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles[J]. J Cell Sci, 2013, 126(24):5553-5565.

[6] Li X, Li X, Lin J, et al. Exosomes Derived From Low-Intensity Pulsed Ultrasound-Treated Dendritic Cells Suppress Tumor Necrosis Factor-Induced Endothelial Inflammation[J]. J Ultrasound Med, 2018, doi: 10.1002/jum.

[7] Zhou Y, Xia L, Lin J, et al. Exosomes in Nasopharyngeal Carcinoma[J]. J Cancer, 2018, 9(5):767-777.

[8] Klinge CM. Non-Coding RNAs in Breast Cancer: Intracellular and Intercellular Communication[J]. Noncoding RNA, 2018, 4(4):40.

[9] Xu Z, Yan Y, Zeng S, et al. Circular RNAs: clinical relevance in cancer[J]. Oncotarget, 2017, 9(1):1444-1460.

[10] Zhong S, Chen X, Wang D, et al. MicroRNA expression profiles of drug-resistance breast cancer cells and their exosomes[J]. Oncotarget, 2016, 7(15):

[11] Tanaka Y, Kamohara H, Kinoshita K, et al. Clinical impact of serum exosomal microRNA-21 as a clinical biomarker in human esophageal squamous cell carcinoma[J]. Cancer, 2013, 119(6):1159-1167.

[12] Hannafon BN, Ding WQ. Functional Role of microRNAs in the Progression of Breast Ductal Carcinoma in situ[J]. Am J Pathol, 2018, pii: S0002-9440(18)

[13] Bei JC, Byrne FL, takenaka K, et al. Analysis of the circular RNA transcriptome in endometrial cancer[J]. Oncotarget, 2018, 5(pp): 5786-5796.

[14] Chin AR, Fong MY, Somlo G, et al. Cross-kingdom inhibition of breast cancer growth by plant miR159[J]. Cell Res, 2016, 26(2):217-228.

[15] Zeng Z, Li Y, Pan Y, et al. Cancer-derived exosomal miR-25-3p promotes pre-metastatic niche formation by inducing vascular permeability and angiogenesis[J]. Nat Commun, 2018, 9(1):5395.

[16] Fujita Y, Kosaka N, Araya J, et al. Extracellular vesicles in lung microenvironment and pathogenesis[J]. Trends Mol Med, 2015, 21(9):533-542.

[17] Tabernero L, Woodman P. Dissecting the role of His domain protein tyrosine phosphatase/PTPN23 and ESCRTs in sorting activated epidermal growth factor receptor to the multivesicular body[J]. Biochem Soc Trans, 2018, 46(5):1037-1046.

[18] Migliano SM, Teis D. ESCRT and Membrane Protein Ubiquitination[J]. Prog Mol Subcell Biol, 2018, 57:107-135.

[19] Stuffers S, Sem WC, Stenmark H, et al. Multivesicular endosome biogenesis in the absence of ESCRTs[J]. Traffic, 2009, 10(7): 925-937.

[20] Dreux M, Garaigorta U, Boyd B, et al. Short range exosomal transfer of viral RNA from infected cells to plasmacytoid dendritic cells triggers innate immunity[J]. Cell Host Microbe, 2012, 12(4): 558-570.

[21] van Niel G, Charrin S, Simoes S, et al. The tetraspanin CD63 regulates ESCRT-independent and -dependent endosomal sorting during melanogenesis[J]. Dev Cell, 2011, 21(4): 708-721.

[22] Frydrychowicz M, Kolecka-Bednarczyk A, Madejczyk M, et al. Exosomes -structure, biogenesis and biological role in non-small-cell lung cancer[J]. Scand J Immunol, 2015, 81(1): 2-10.

[23] Ludwig A K, Giebel B. Exosomes: small vesicles participating in intercellular communication[J]. Int J Biochem Cell Biol, 2012(44):11-15.

[24] Wang S, Cesca F, Loers G, et al. Synapsin I is an oligomannose carrying glycoprotein, acts as an oligomannose-binding lectin, and promotes neurite outgrowth and neuronal survival when released via gliaderived exosomes[J]. J Neuroscience, 2011, 31(20):7275-7290.

[25] Vlassov AV, Magdaleno S, Setterquist R, et al. Exosomes: current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials[J]. Biochim Biophys Acta, 2012, 1820(7):940-948.

[26] Clotilde T, Muriel B, Philippe V, et al. Proteomic analysis of dendritic cell-derived exosomes: a secreted subcellular compartment distinct from apoptotic vesicles[J]. J Immunol, 2001, 166(12):7309-7318.

[27] Hao S, Bai O, Li F, et al. Mature dendritic cells pulsed with exosomes stimulate efficient cytotoxic T-lymphocyte responses and antitumour immunity[J]. Immunology, 2006, 10(1111):1365-2567.

[28] Alvarez-Erviti L, Seow Y, Yin H, et al. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes[J]. Nature Biotechnology, 2011, 29(4):341-345.

[29] Qi H, Liu C, Long L, et al. Blood exosomes endowed with magnetic and targeting properties for cancer therapy[J]. ACS Nano, 2016, 10(3):3323.

[30] Chen Z, Fillmore CM, Hammerman PS, et al. Non-small-cell lung cancers: a heterogeneous set of diseases[J]. Nat Rev Cancer, 2014, 14(8): 535-546.

[31] Ueda K, Ishikawa N, Tatsuguchi A, et al. Antibody-coupled Monolithic silica microtips for highthroughput molecular profiling of circulating exosomes[J]. Sci Rep, 2014, 4:6232.

[32] Vanni I, Alama A, Grossi F, et al. Exosomes: a new horizon in lung cancer[J]. Drug Discov Today, 2017(17):30110-30115.

[33] Melo SA, Luecke LB, Kahlert C, et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer[J]. Nature, 2015, 523(7559):177-182.

[34] Que R, Ding G, Chen J, et al. Analysis of serum exosomal microRNAs and clinicopathologic features of patients with pancreatic adenocarcinoma[J]. World J Surg Oncol, 2013, 11(1):1-9.

[35] Tauro BJ, Greening DW, Mathias RA, et al. Two distinct populations of exosomes released from LIM1863 colon carcinoma cell-derived organoids[J]. Mol Cell Proteomics, 2013, 12(3):587-598.

[36] Ogata-Kawata H, Izumiya M, Kurioka, et al. Circulating exosomal microRNAs as biomarkers of colon cancer[J]. PLoS One, 2014, 9(4):e92921.

[37] Hu D, Zhan Y, Zhu K, et al. Plasma Exosomal Long Non-Coding RNAs Serve as Biomarkers for Early Detection of Colorectal Cancer[J]. Cell Physiol Biochem, 2018, 51(6):2704-2715.

[38] Szajnik M, Derbis M, Lach M, et al. Exosomes in plasma of patients with ovarian carcinoma: potential biomarkers of tumor progression and response to therapy[J]. Gynecology & Obstetrics, 2013, S4-003.

[39] Ying X, Wu Q, Wu X, et al. Epithelial ovarian cancer-secreted exosomal miR-222-3p induces polarization of tumor-associated macrophages[J]. Oncotarget, 2016, 7(28):43076-43087.

[40] Li Z, Ma YY, Wang J, et al. Exosomal microRNA-141 is upregulated in the serum of prostate cancer patients[J]. Onco Target Ther, 2016, 9:139-148.

[41] Zijlstra C, Stoorvogel W. Prostasomes as a source of diagnostic biomarkers for prostate cancer[J]. J Clin Invest, 2016, 126(4):1144-1151.

[42] Beckham CJ, Olsen J, Yin PN, et al. Bladder cancer exosomes contain EDIL-3/Del1 and facilitate cancer progression[J]. The Journal of Urology, 2014, 192(2):583-592.

[43] Long JD, Sullivan TB, Humphrey J, et al. A non-invasive miRNA based assay to detect bladder cancer in cell-free urine[J]. Am J Transl Res, 2015, 7(11):

[44] Zheng R, Du M, Wang X, et al. Exosome-transmitted long non-coding RNA PTENP1 suppresses bladder cancer progression[J]. Mol Cancer, 2018, 17(1):143.

[45] Halvaei, S, Daryani, S, Eslami-S Z, et al. Exosomes in Cancer Liquid Biopsy: A Focus on Breast Cancer[J]. Mol. Ther. Nucleic Acids, 2018, 10:131-141.

[46] Li Y, Zheng Q, Bao C, et al. Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis[J]. Cell Res, 2015, 25(8):

[47] Xu H, Gong Z, Shen Y, et al. Circular RNA expression in extracellular vesicles isolated from serum of patients with endometrial cancer[J]. Epigenomics,

2018, 10(2):187-197.

[48] Wang H, Hou L, Li A, et al. Expression of serum exosomal microRNA-21 in human hepatocellular carcinoma[J]. Biomed Res Int, 2014, 2014:864894.

[49] Fujiwara T, Uotani K, Yoshida A, et al. Clinical significance of circulating miR-25-3p as a novel diagnostic and prognostic biomarker in osteosarcoma[J]. Oncotarget, 2017, 8(20):33375-33392.

[50] Zhang Y, Luo CL, He BC, et al. Exosomes derived from IL-12-anchored renal cancer cells increase induction of specific antitumor response in vitro: a novel vaccine for renal cell carcinoma[J]. Int J Oncol, 2010, 36(1):133-140.

Note: Figure translations are in progress. See original paper for figures.