Analytical and Bioanalytical Chemistry
https://doi.org/10.1007/s00216-019-02211-4

RESEARCH PAPER

Rapid and sensitive exosome detection with CRISPR/Cas12a

Xianxian Zhao[1] · Wenqing Zhang[1] · Xiaopei Qiu[1] · Qiang Mei[3] · Yang Luo[2] · Weiling Fu[1]

Received: 10 August 2019 / Revised: 8 October 2019 / Accepted: 10 October 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

Numerous studies have shown that exosomes are closely related to the pathogenesis of various diseases, especially cancers. Therefore, a rapid and sensitive method for exosome detection will be of great importance for the diagnosis and prognosis of diseases. We report here a method for exosome detection based on the CD63 aptamer and clustered regular interspaced short palindromic repeats (CRISPR)/Cas12a system. This method consists mainly of exosomal membrane protein recognition based on the CD63 aptamer and signal amplification based on CRISPR/Cas12a. The CD63 aptamer, as an easily adaptable nucleic acid strand, is responsible for the conversion of the amounts of exosomes into nucleic acid detection, whereas CRISPR/Cas12a is responsible for highly specific nucleic acid signal amplification. The detection range of the method was determined as 3×10^3–6×10^7 particles per microliter. Additionally, we successfully applied this method to detect exosomes in clinical samples from both healthy individuals and patients with lung cancer, and the results were highly consistent with those obtained by nanoparticle tracking analysis. In general, this method provides a highly sensitive and specific method for the detection of exosomes and offers an avenue toward future exosome-based diagnosis of diseases.

Keywords CRISPR/Cas12a · Exosome · Aptasensor · CD63

Introduction

Exosomes are small vesicles with a diameter of about 30–50 nm and a density of 1.13–1.21 g/ml that contain various fragments, such as RNA, protein, microRNA, and DNA fragments [1–3]. Exosomes are released from cells by fusion of multivesicular bodies with the plasma membrane and thus can be found in almost all body fluids, including blood [4], saliva [5], urine [6], and milk [7]. Proteins play critical roles in maintaining cell recognition and structural reinforcement of the exosomal membrane. Recent reports revealed that a group of proteins are overexpressed on various cell-derived exosomes, such as cytoplasmic proteins, transmembrane proteins, and Rab [8]. Among them, CD9 and CD63 are universal transmembrane proteins frequently used as exosome-specific markers for identification [9, 10], whereas the others are tissue-specific proteins that are closely related to various biological functions [11]. Participating in intercellular communication is one of the most important functions of exosomes [12, 13]. Exosomes act on target cells in different ways to achieve information transmission between cells and produce a series of physiological or pathological reactions, such as angiogenesis, immune regulation, and antigen presentation [1, 14, 15]. Therefore, exosomes, as thumbnails of cells, are a promising biomarker for the noninvasive diagnosis and treatment of diseases.

The value of exosomes for medical research has prompted the search for a fast and efficient method of detection. Although traditional immunoaffinity-based techniques such as western blotting, enzyme-linked immunosorbent assay,

Xianxian Zhao, Wenqing Zhang, and Xiaopei Qiu contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00216-019-02211-4) contains supplementary material, which is available to authorized users.

✉ Yang Luo
luoy@cqu.edu.cn

✉ Weiling Fu
fwlamu@163.com

[1] Department of Clinical Laboratory, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing 400038, China

[2] Key Laboratory for Biorheological Science and Technology of Ministry of Education, State and Local Joint Engineering Laboratory for Vascular Implants, Bioengineering College of Chongqing University, Chongqing 400044, China

[3] Department of Pathology, 922th Hospital of the People's Liberation Army, Hengyang 421002, Hunan, China

Published online: 02 January 2020

and flow cytometry have been used for exosome analysis, they are limited by some shortcomings [2, 16]. Flow cytometry is costly and heavily dependent on instrument support, whereas both western blotting, and enzyme-linked immunosorbent assay have intrinsic sensitivity limitations. These immunoaffinity-based approaches can identify typical markers of exosomal proteins but are also greatly limited by inconsistency between batches [17, 18]. Furthermore, various biosensors were also reported to achieve ultrasensitive detection of exosomes through membrane proteins (CD63 and CD9), including by colorimetry, immunofluorescence, surface-enhanced Raman scattering, electrochemistry, and microfluidics [19–21]. Nevertheless, they are still limited by complicated procedures and the requirement for special instruments.

Aptamers are single-stranded oligonucleotide fragments (usually 15–80 nucleotides) screened from a random library using systematic evolution of ligands by exponential enrichment. They can be folded to form a uniquely stable three-dimensional structure that specifically binds to multiple targets by electrostatic interactions, hydrogen bonding, van der Waals forces, alkali stacking, or multiple combinations of forces [22, 23]. Aptamers are used as ideal recognition ligands in biological assays because of their variable conformation and are referred to as "chemical antibodies," suggesting excellent antigen recognition capabilities comparable to those of antibodies [24, 25]. Differently, aptamers can be designed for signal amplification, and thus exhibit a wide range of applications while ensuring highly specific protein recognition [26, 27]. Although various isothermal nucleic acid amplification techniques, such as rolling circle amplification, strand displacement amplification, and recombinase polymerase amplification, have been reported for signal amplification, they have frequently encountered inevitable shortcomings, such as nonspecific amplification and complicated procedures [28]. Clustered regular interspaced short palindromic repeats (CRISPR), the most widely used gene editing system, provides a new idea for signal amplification [29]. CRISPR/Cas12a has attracted much attention because of its *cis*-cleavage and *trans*-cleavage features [30]. On the basis of this, DNA endonuclease targeted CRISPR *trans* reporter (DETECTR) has been reported to achieve attomole-level single-base detection of nucleic acid molecules [31].

Here we report a rapid and sensitive exosome detection technique based on CD63 aptamer and *trans* cleavage by CRISPR/Cas12a. In our design, the CD63 aptamer, partially blocked by complementary DNA strands (blocker), is used to capture CD63-bearing exosomes. The combination of exosomes and CD63 aptamer triggers a conformational change of CD63 aptamer to release the blocker. The released blocker can be recognized by CRISPR/Cas12a and triggers *trans* cleavage toward a TaqMan probe reporter. As a result, reporter probes labeled with fluorochrome (Cy3) and a corresponding quenching group (Black Hole Quencher) at both terminals are cut, thereby generating a fluorescent signal. The resulting fluorescent signal is positively correlated with the exosomes captured by the magnetic beads. This strategy has been proven to be capable of detecting exosomes over four orders of magnitude and has been successfully applied in the detection of exosomes released from cultured cells and the detection of plasma exosomes.

Experimental

Reagents and materials

All of the oligonucleotides used in this work were purchased from Sangon Biological Engineering Technology & Services Co. (Shanghai, China), including aptamer 1 and aptamer 2, which were designed according to relevant references [19, 32], blockers, reporters, and Apt-R. The sequences are listed in Table S1. Dynabeads MyOne streptavidin magnetic beads (SMBs), Dulbecco's modified Eagle medium, and fetal bovine serum were purchased from Thermo Fisher Scientific (USA). The A549 cell line was obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Radioimmunoprecipitation assay lysis buffer, trypsin–EDTA, and penicillin–streptomycin were purchased from Solarbio Science and Technology Co. (Beijing, China). Tween 20 was purchased from Aladdin Biochemical Technology Co. (Shanghai, China). Bovine serum albumin and tris(2-carboxyethyl)phosphine were purchased from Sigma-Aldrich (St Louis, MO, USA). The 10-kDa ultrafiltration device and 0.22-μm filters were purchased from Millipore (Bedford, MA, USA). Cas12a enzymes were purchased from Inovogen Technology Co. (Beijing, China). The purified recombinant human CD9 and CD63 proteins were purchased from Abcam (Cambridge, MA, USA).

Instrumentation

A549 cells were cultured in a carbon dioxide incubator (Galaxy 170S, Eppendorf, Germany). Fetal bovine serum was ultracentrifuged with a CP80MX ultracentrifuge with a P40ST rotor (Hitachi Aloka Medical, Tokyo, Japan). The concentration and the size of the exosomes were measured by ZetaView (Particle Metrix, Meerbusch, Germany). Transmission electron microscopy imaging of exosomes was performed with a JEM-2100 instrument (JEOL, Japan). The gels were visualized with a Bio-Rad molecular imager (Bio-Rad, Beijing, China) and a gel image system (Tanon, Shanghai, China). All the mixing operations were conducted with a HulaMixer sample mixer (Thermo Fisher Scientific, Waltham, MA, USA).

Cell culture, exosome isolation, and exosome characterization

A549 cells were incubated in a humidified atmosphere containing 5% CO_2 at 37 °C and were used as the source of exosomes. A549 cells were rinsed twice with phosphate-buffered saline (PBS) and transferred into serum-free medium for 48 h when the cells had grown to more than 50% confluence. Exosomes secreted by A549 cells were obtained by ultracentrifugation of cell culture supernatant. The ultracentrifugation steps were as reported previously [33, 34]. Two steps were included to remove cellular debris and the larger microvesicles, including a first centrifugation at 2000*g* for 10 min and a second centrifugation at 10,000*g* for 60 min. The exosome-containing supernatant was then filtered by a 0.22-μm filter, followed by ultracentrifugation at 100,000*g* for 150 min at 4 °C to precipitate exosomes. The sediment finally obtained was washed with 1× PBS and resuspended in 10 mM PBS for nanoparticle tracking analysis (NTA) and further use.

Immobilization of Apt63 probe on the surfaces of SMBs

The surfaces of SMBs were labeled with Apt63 as follows, First, 5 μl CD63 aptamer and 7.5 μl blocker were mixed with 37.5 μl 1× PBS and prehybridized with a PCR machine (90 °C for 5 min, cooled to 4 °C at a speed of 4 °C/min) to form Apt63. Then SMBs (10 μl, 10 mg/ml) were washed three times with washing buffer and diluted to 1 mg/ml. Apt63 (10 μl) was added to the diluted SMB solution and mixed for 30 min at room temperature, followed by magnetic separation of the Apt63-labeled SMBs. The Apt63–SMB complexes obtained were washed twice with washing solution. (All the operations described in the section were in accordance with the Dynabeads™ MyOne™ streptavidin T1 user guide).

Exosome detection procedure

Exosome detection consisted of two procedures in this method. The first procedure is the capture of exosomes by Apt63-SMBs. Cell culture supernatant (300 μl) was obtained from cultured A549 cells and mixed with Apt63–SMB complex solution for 60 min. The Apt63–SMB complexes were then collected with the help of a magnet. The supernatant obtained at this time contained blocker released when the CD63 aptamer was bound to CD63. The second procedure is signal amplification based on the released blocker collected from the supernatant. In this step, 5 μl Cas12a enzymes (1 μM) and 5 μl single guide RNA (1 μM) were added to the supernatant together to form a CRISPR/Cas12a–single guide RNA complex to recognize the blocker and arbitrarily cut reporter probes added together with Cas12a enzymes (1 μM). After 15 min of incubation, the intensity of the generated fluorescent signal was measured with a fluorescence spectrophotometer (Hitachi, Tokyo, Japan).

Results and discussion

Principle and design of the approach

Figure 1 demonstrates the mechanism of the strategy, which contains two parts: CD63-aptamer-based exosome capture and separation, and blocker-based signal amplification. Inspired by the fact that CD63 aptamers are shared by different cell-derived exosomes, the CD63 aptamer was selected to specifically capture exosomes. On this basis, we designed capture probe Apt63, which consists of a biotin-terminated CD63 aptamer and a blocker that is partially complementary to CD63 aptamer. Apt63 is attached to the SMBs through the interaction between biotin and streptavidin. When exosomes are present, the Apt63–SMB complex captures exosomes, leading to a conformational change of the CD63 aptamer that results in release of the blocker. After removal of the remaining Apt63–SMB complexes and captured exosomes by a magnet, the blockers in the supernatant could be recognized by the CRISPR/Cas12a system and triggered *trans*-cleavage activity. As a result, the reporter probes labeled with Cy3 and Black Hole Quencher at both terminals, respectively, were cut to produce a fluorescent readout. Because each activated CRISPR/Cas12a molecule could cleave amounts of reporters (500 nM) in 20 min. the original detected signals could be promptly amplified. Thus, the proposed method could be used as a rapid and sensitive exosome detection approach.

Investigation of exosome capture process

We prepared lung cancer exosomes from A549 cells using a standard ultracentrifugation method and extensively evaluated the obtained exosomes. The transmission electron microscopy images (Fig. 2a) clearly showed a group of round and partially collapsed structures (diameters of approximately 110 nm) that have sizes comparable to those of exosomes reported in the literature [35, 36]. NTA demonstrated a relatively homogeneous population with diameters ranging from 30 to 451 nm, with a peak of 104 nm and a calculated mean of 117.5 nm (Fig. 2b). Western blotting showed that several representative proteins (HSP70, HSP90, and TSG101) were highly expressed both in lung cancer exosomes and in A549 cells, and the levels of several exosomal specific markers (CD9, CD63, and CD81) were higher in exosomes than in A549 cells (Fig. 2c), whereas no obvious proteins were observed in the negative control group (fetal bovine serum-free RPMI 1640 medium).

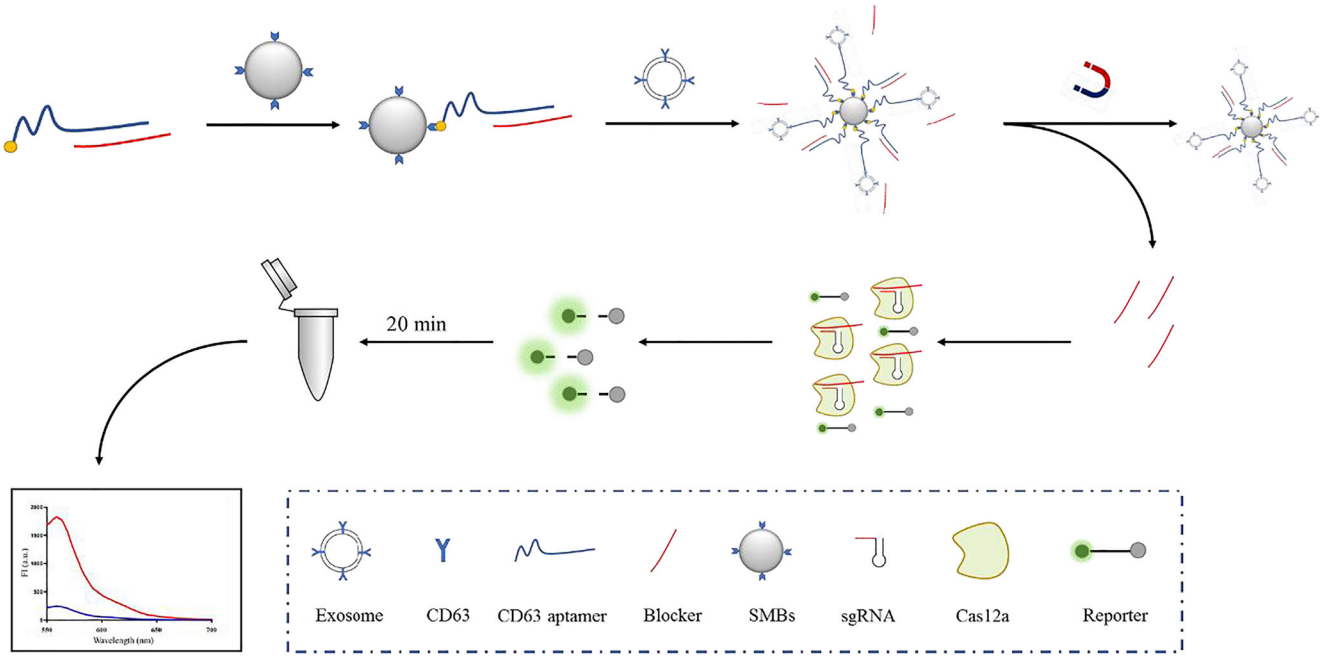


Fig. 1 Clustered regular interspaced short palindromic repeats (CRISPR)/Cas12a-based signal amplification for exosome detection. a.u. arbitrary units, FI fluorescence intensity, sg RNA single guide RNA, SMB streptavidin molecular bead

Furthermore, to evaluate the release efficiency of blocker within Apt63 in the presence of exosomes, aptamer 1–blocker 1, aptamer 2–blocker 2, and aptamer 2–blocker 3 were designed according to the relevant reference [19, 32]. Among them, aptamer 1 and aptamer 2 were designed by our adding TTTTTT at the 3′ end of the 32-mer CD63 aptamer for immobilization of SMBs. Additionally, we further modified aptamer 1 by adding a five-base sequence (ATATA) that can hybridize with blocker 1 at the 5′ end to make the aptamer 1–blocker 1 complex stabler. In an experiment validating the affinity of the modified aptamer 1 for binding with CD63, we compared the original 32-mer CD63 DNA aptamer with the five-base-modified aptamer (aptamer 1) in binding with CD63-positive exosomes and found these two aptamers achieved identical results in binding CD63-positive exosomes (Fig. S1). Finally, blocker 1, blocker 2, and blocker 3 were designed with 12-base complementarity to aptamer 1 and aptamer 2 at different positions. Blocker 2 has an additional three-base tail to show the difference with blocker 3, which has the same hybridization position for aptamer 2 (Fig. 2d). Figure 2e demonstrates that the fluorescent signal ratio of aptamer 1–blocker 1 was significantly higher than that of aptamer 2–blocker 2 and aptamer 2–blocker 3 in the presence of exosomes. This is probably because the stabilities of aptamer 2–locker 2 and aptamer 2–blocker 3 are too high to be disrupted by the interaction between CD63 and aptamer 2. Therefore, blocker 1 and aptamer 1 were selected to be assembled into Apt63. In addition, we investigated the recognition specificity between Apt63 and CD6. First, we designed Apt-R, which is composed of a CD63-aptamer-like sequence and a random blocker sequence (blocker-R) to determine the interaction between the Apt63 probe and CD63. Figure 2f demonstrates a relatively higher fluorescent signal when CD63 was mixed with Apt63 than with Apt-R (1153 ± 43.54 vs 184 ± 12.54, emission wavelength 560 nm), suggesting a specific interaction between CD63 and Apt63. A further experiment showed that CD63–Apt63 hybridization yielded a 6.8 times higher fluorescent signal than CD9-Apt63 hybridization (1153 ± 43.54 vs 168 ± 16.23, emission wavelength 560 nm), validating the high specificity of Apt63 in recognizing CD63. Similar results were also shown in a complex environment (Fig. S2). Furthermore, we also explored the stability of Apt63 in a complex environment to determine its feasibility in detection of plasma exosomes. Figure 2g shows that the fluorescent signal was higher in cell culture supernatant solution containing exosomes than in a exosome-free complex environment (Dulbecco's modified Eagle medium and 0.1% bovine serum albumin) (1598.74 vs 497.21 and 389.54, emission wavelength 560 nm), revealing that the Apt63–blocker complex exhibited satisfactory stability by showing negligible probe blocker 1 release in the absence of exosomes. Meanwhile, the efficiency of capturing exosomes by Apt63–SMB complexes was determined as 85% by our comparing the amounts of exosomes before and after Apt63–SMB capture using NTA (Fig. S3).

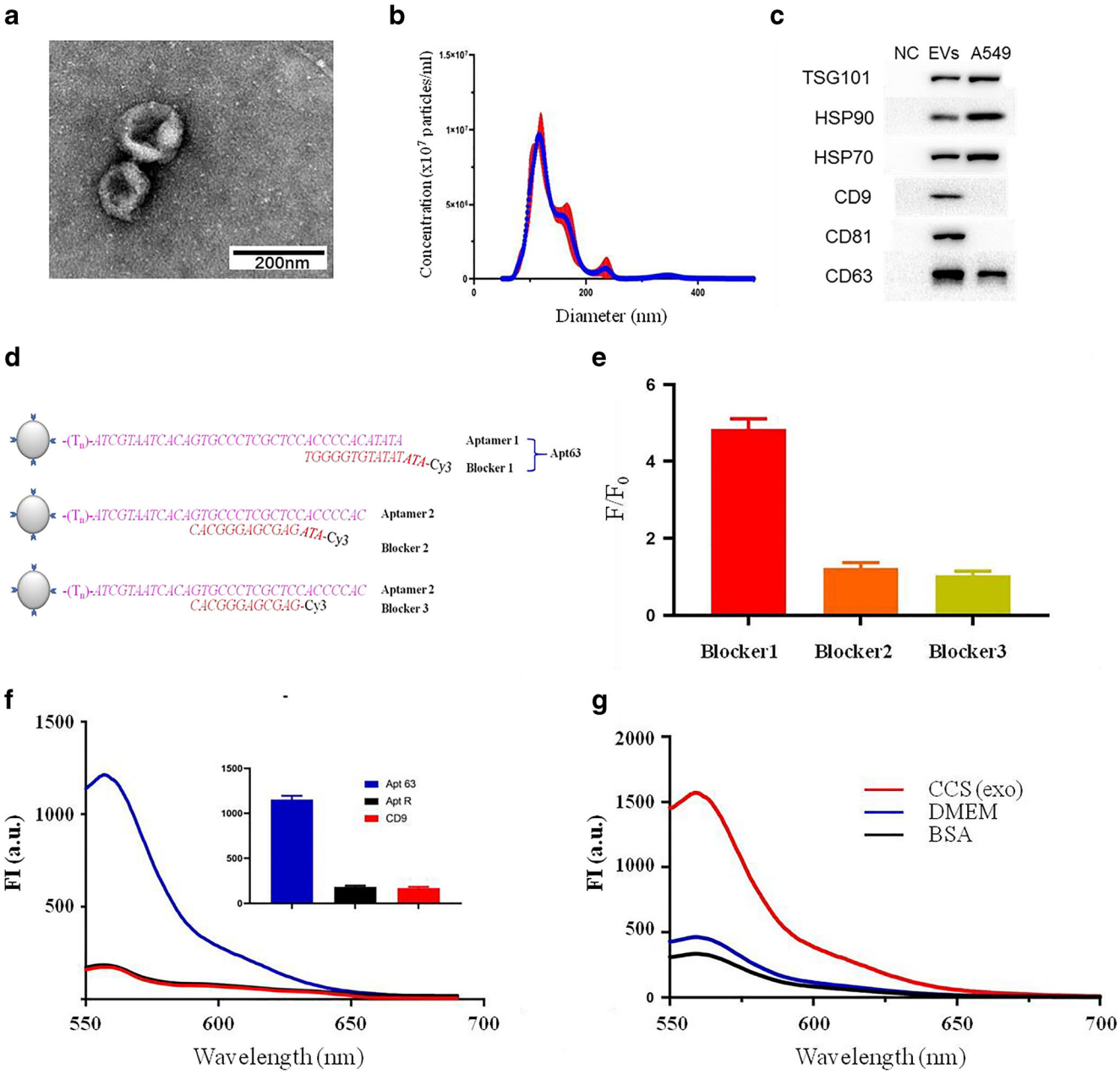


Fig. 2 Investigation of exosome capturing capability. **a** Transmission electron microscopy image of exosomes. Scale bar 200 nm. **b** Concentrations and size distributions of extracted exosomes determined by nanoparticle tracking analysis. Nanoparticle tracking analysis histograms represent the mean size (blue line) of exosomes from three parallel samples; the red area denotes the overall distribution of all samples tested. **c** Western blotting analysis of several representative proteins in the negative control (NC; fetal bovine serum-free RPMI 1640 medium), exosomes (EVs), and A549 cells. **d** Optimization of the sequences of blockers. Blocker 2 and blocker 3 share the same complementary sequences for aptamer 2, and blocker 2 has an additional six nucleotides. **e** F/F_0 ratios of three blockers in the presence of exosomes, where F represents the fluorescence intensity of exosomes and F_0 represents the background signal. **f** Fluorescence spectra of Apt-R and Apt63 in the presence of CD63 and of Apt63 in the presence of CD9. The inset shows the fluorescence intensity of the three groups (emission wavelength 560 nm). Data are represented as the mean ± standard deviation (n = 3). **g** Fluorescence spectra of exosomes in complex solutions. Exosome concentration, 6×10^5 particles per microliter. a.u. arbitrary units, BSA bovine serum albumin, CCS cell culture supernatant, DMEM Dulbecco's modified Eagle medium, exo exosomes, FI fluorescence intensity

Investigation of the feasibility of CRISPR/Cas12a

We first explored the *trans*-cleavage activity of CRISPR/Cas12a on reporter probes when recognizing the blocker. The result showed that blocker 1 could induce a significant fluorescent signal increase, suggesting that blocker 1 can be recognized by the CRISPR/Cas12a system and so trigger *trans* cleavage of the reporter (Fig. 3a). Furthermore, we

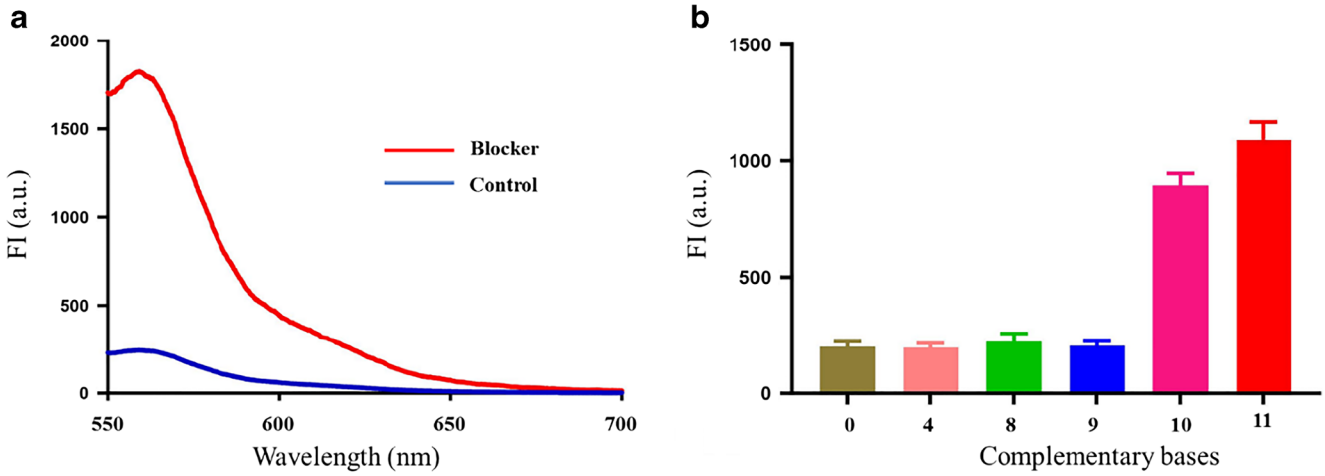


Fig. 3 Characterization of *trans* cleavage by the clustered regular interspaced short palindromic repeats (CRISPR)/Cas12a system. **a** *Trans* cleavage by CRISPR/Cas12a on reporter probes in the presence and absence (control) of blocker. **b** The ability to activate *trans* cleavage of six sequences (mat11, mat10, mat9, mat8, mat4, and mat0) that had 11,10, 9, 8, 4, and 0 bases identical to the bases of blocker 1. a.u. arbitrary units, FI fluorescence intensity,

investigated the ability to active *trans* cleavage of six sequences (mat11, mat10, mat9, mat8, mat4, and mat0) that had 11,10, 9, 8, 4, and 0 bases identical with the bases of blocker 1. Figure 3b reveals the presence of mat10 could trigger *trans* cleavage by CRISPR/Cas12a on fluorescent probes, while the other four sequences (mat0, mat4, mat8, and mat9) could not, suggesting that fully complementarity of the blocker with single guide RNA for ten bases in the CRISPR/Cas12a system was necessary for signal amplification, ensuring highly specific blocker recognition (as only 12 bases are totally complementary to CD63 aptamer in blocker 1).

Optimization of experimental conditions

We successively optimized the concentration of Apt63 as a label on SMBs, the exosome-capture time, and the *trans*-cleavage time of CRISPR/Cas12a to achieve better exosome detection performance. As an important part of this approach, the Apt63–SMB complex is not only involved in the capture of exosomes but also plays a crucial role in converting exosome capture to signal amplification. The concentration of Apt63 linked to SMBs is an essential factor for exosome capture. Therefore, we first investigated the efficiency of exosome capture by SMBs linked to Apt63 in different concentrations by detecting the Cy3 label on the blocker. Figure 4a shows F/F_0 gradually increased as the concentration of Apt63 increased and reached a peak at 0.5 μM. Therefore, the most appropriate Apt63 concentration was determined to be 0.5 μM. We then studied the exosome-capture time. We found that F/F_0 increased significantly between 0 min to 60 min, but the amount of increase dropped after 60 min. Therefore, 60 min was optimal for the magnetic beads to capture the exosomes (Fig. 4b). Finally, the reaction time of CRISPR/Cas12a was explored. We found that the fluorescence intensity gradually became stable after 20 min. Therefore, we chose 20 min as the optimized reaction time (Fig. 4c). Under the optimized conditions, the fluorescence intensity was linearly related to the logarithmic amount of exosomes ranging from 10^3 to 10^7 particles per microliter (Fig. 4d). The correlation equation between the fluorescence intensity and the amount of exosomes was determined as $y = 592.4 \lg c_{exo} - 1588$ ($R^2 = 0993$). Compared with previously reported immunoaffinity-based exosome detection approaches [19, 21, 37], this method has a relatively consistent detection dynamic range from 10^3 to 10^7 particles per microliter.

Application of the method in clinical samples

To further study the clinical applications of the method for the detection of exosomes from clinical samples, blood samples from patients with lung cancer and healthy individuals were collected. Plasma was obtained from blood samples after centrifugation at 5000*g* for 10 min and filtered by a 0.22-μm filter. Eventually, four serum samples were collected. Then exosomes in each sample were detected separately by the proposed method and NTA. The pretreated serum samples were further ultracentrifuged and quantified by NTA, whereas the same serum samples without ultracentrifugation were detected by the proposed method and the exosomal concentration was calculated by the calibration equation given in the previous section and illustrated in Fig. 4d. As shown in Fig. 5, the amounts of exosomes detected by this method were highly consistent with the NTA results, demonstrating that this method has high application potential in the detection of clinical specimens.

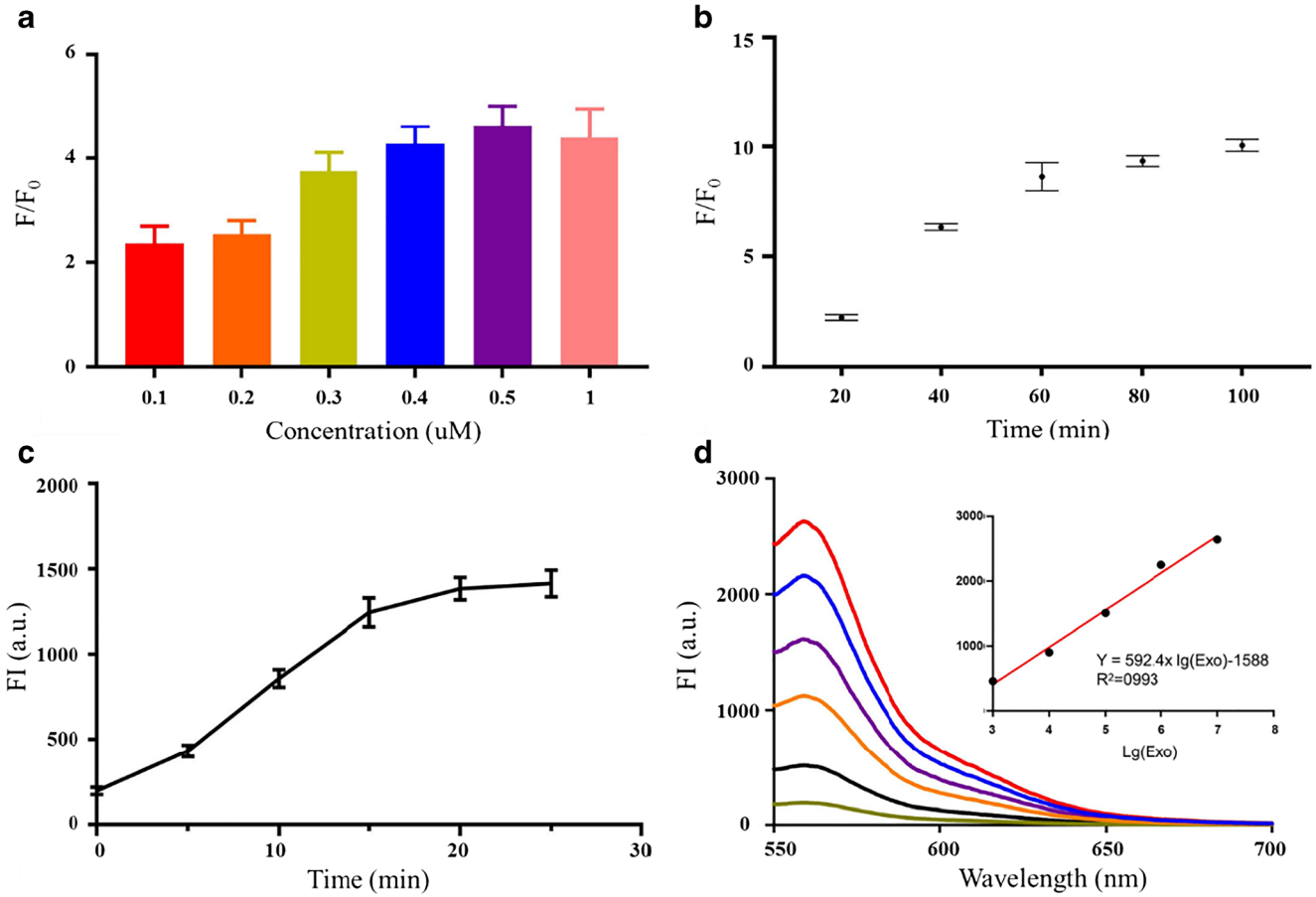


Fig. 4 Optimization of experimental conditions. **a** Fluorescence ratio (F/F_0) of different concentrations of Apt63 attached to streptavidin molecular beads (SMBs). **b** Optimization of the time for Apt63–SMB complexes to capture exosomes. **c** Optimization of the time for clustered regular interspaced short palindromic repeats (CRISPR)/Cas12a *trans* cleavage. **d** Sensitivity of the method for exosome detection. The concentration of exosomes was determined by nanoparticle tracking analysis as 6×10^7, 6×10^6, 6×10^5, 6×10^4, 3×10^3, and zero particles per microliter. $n = 3$. a.u. arbitrary units, exo exosomes, FI fluorescence intensity.

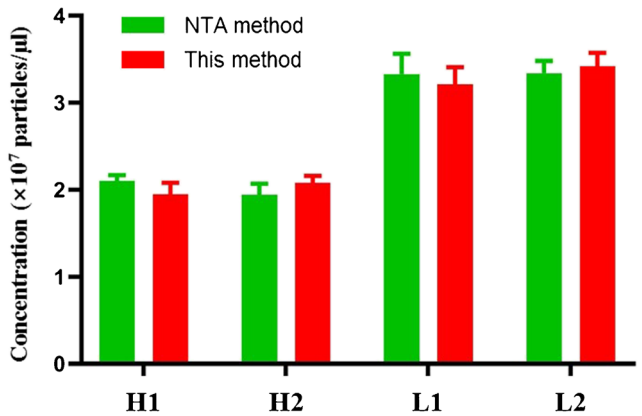


Fig. 5 Application of the method in clinical samples. Detection of exosomes by nanoparticle tracking analysis (NTA) and the proposed method from healthy individuals (H1, H2) and patients with lung cancer (L1, L2). The green column is for the NTA method and the red column is for the clustered regular interspaced short palindromic repeats (CRISPR)/Cas12 method. Data are represented as the mean ± standard deviation ($n = 3$)

Limitation of the method

Although the method demonstrated satisfactory performance in both laboratory and clinical applications, some deficiencies potentially existed. Since not all exosomes have overexpressed CD63, this method based on CD63 aptamer for exosome capture might have the potential risk of capturing CD63-negative exosomes. Additionally, the discrepant expression level of CD63 on exosomal membrane from different patients might lead to analytical bias.

Conclusions

We have developed a rapid and sensitive exosome detection method by integrating CD63 aptamer for exosome capture and CRISPR/Cas12a for signal amplification. The proposed method stands out for its simple component requirements for signal amplification and high specificity ensured by CRISPR/Cas12a [38, 39]. As a result, this method achieved a detection

range of 10^3 to 10^7 particles per microliter without the use of ultracentrifugation and can complete exosome detection within 2 h. The method, in which aptamer is used to convent exosome capture events into single-stranded DNA probe detection, provides a new idea for the application of CRISPR/Cas12a in exosome detection.

Acknowledgements The authors thank the volunteers who provided the clinical samples for this study.

Funding This work was partially supported by grants from the National Natural Science Foundation of China (81772290, 81601854, and 81572079).

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethics approval Informed consent was obtained from all participants in the clinical trial and the protocols were approved by the First Affiliated Hospital of Third Military Medical University (Army Medical University) Ethics Committee.

References

1. Alharbi M, Lai A, Guanzon D, Palma C, Zuniga F, Perrin L, et al. Ovarian cancer-derived exosomes promote tumor metastasis in vivo an effect modulated by the invasiveness capacity of their originating cells. Clin Sci (Lond). 2019;133(13):1401–19.
2. Rokad D, Jin H, Anantharam V, Kanthasamy A, Kanthasamy AG. Exosomes as mediators of chemical-induced toxicity. Curr Environ Health Rep. 2019;6(3):73–9.
3. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol. 2013;200(4):373–83.
4. Wu M, Ouyang Y, Wang Z, Zhang R, Huang PH, Chen C, et al. Isolation of exosomes from whole blood by integrating acoustics and microfluidics. Proc Natl Acad Sci U S A. 2017;114(40):10584–9.
5. Sun Y, Liu S, Qiao Z, Shang Z, Xia Z, Niu X, et al. Systematic comparison of exosomal proteomes from human saliva and serum for the detection of lung cancer. Anal Chim Acta. 2017;982:84–95.
6. Street JM, Koritzinsky EH, Glispie DM, Yuen PST. Urine exosome isolation and characterization. Methods Mol Biol. 2017;1641:413–23.
7. Wu Q, Li J, Li Z, Sun S, Zhu S, Wang L, et al. Exosomes from the tumour-adipocyte interplay stimulate beige/brown differentiation and reprogram metabolism in stromal adipocytes to promote tumour progression. J Exp Clin Cancer Res. 2019;38(1):223.
8. Xu H, Jia S, Xu H. Therapeutic potential of exosomes in autoimmune diseases. Clin Immunol. 2019;205:116-24.
9. Oksvold MP, Neurauter A, Pedersen KW. Magnetic bead-based isolation of exosomes. Methods Mol Biol. 2015;1218:465–81.
10. Cavallaro S, Horak J, Haag P, Gupta D, Stiller C, Sahu SS, et al. Label-free surface protein profiling of extracellular vesicles by an electrokinetic sensor. ACS Sens. 2019;4(5):1399–408.
11. Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol. 2002;2(8):569–79.
12. Su SA, Xie Y, Fu Z, Wang Y, Wang JA, Xiang M. Emerging role of exosome-mediated intercellular communication in vascular remodeling. Oncotarget. 2017;8(15):25700–12.
13. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007;9(6):654–9.
14. Al-Sowayan BS, Al-Shareeda AT, Al-Hujaily EM. Exosomes, cancer's little army. Stem Cell Investig. 2019;6:9.
15. Wu Q, Zhou L, Lv D, Zhu X, Tang H. Exosome-mediated communication in the tumor microenvironment contributes to hepatocellular carcinoma development and progression. J Hematol Oncol. 2019;12(1):53.
16. Wang Y, Liu Z, Wang X, Dai Y, Li X, Gao S, et al. Rapid and quantitative analysis of exosomes by a chemiluminescence immunoassay using superparamagnetic iron oxide particles. J Biomed Nanotechnol. 2019;15(8):1792–800.
17. Khodashenas S, Khalili S, Forouzandeh Moghadam M. A cell ELISA based method for exosome detection in diagnostic and therapeutic applications. Biotechnol Lett. 2019;41(4-5):523–31.
18. Khongkow M, Yata T, Boonrungsiman S, Ruktanonchai UR, Graham D, Namdee K. Surface modification of gold nanoparticles with neuron-targeted exosome for enhanced blood-brain barrier penetration. Sci Rep. 2019;9(1):8278.
19. Gao ML, He F, Yin BC, Ye BC. A dual signal amplification method for exosome detection based on DNA dendrimer self-assembly. Analyst. 2019;144(6):1995–2002.
20. Houalla R, Devaux F, Fatica A, Kufel J, Barrass D, Torchet C, et al. Microarray detection of novel nuclear RNA substrates for the exosome. Yeast. 2006;23(6):439–54.
21. Wang Q, Zou L, Yang X, Liu X, Nie W, Zheng Y, et al. Direct quantification of cancerous exosomes via surface plasmon resonance with dual gold nanoparticle-assisted signal amplification. Biosens Bioelectron. 2019;135:129–36.
22. Wang X, Hao Z, Olsen TR, Zhang W, Lin Q. Measurements of aptamer-protein binding kinetics using graphene field-effect transistors. Nanoscale. 2019;11(26):12573–81.
23. Albanese CM, Suttapitugsakul S, Perati S, McGown LB. A genome-inspired, reverse selection approach to aptamer discovery. Talanta. 2018;177:150–6.
24. Kinghorn AB, Fraser LA, Lang S, Shiu SCC, Tanner JA. Aptamer bioinformatics. Int J Mol Sci. 2017;18(12).
25. Verdian A, Fooladi E, Rouhbakhsh Z. Recent progress in the development of recognition bioelements for polychlorinated biphenyls detection: antibodies and aptamers. Talanta. 2019;202:123–35.
26. Yan J, Xiong H, Cai S, Wen N, He Q, Liu Y, et al. Advances in aptamer screening technologies. Talanta. 2019;200:124–44.
27. Zhang Y, Lai BS, Juhas M. Recent advances in aptamer discovery and applications. Molecules. 2019;24(5).
28. Mayboroda O, Katakis I, O'Sullivan CK. Multiplexed isothermal nucleic acid amplification. Anal Biochem. 2018;545:20–30.
29. Li Y, Li S, Wang J, Liu G. CRISPR/Cas systems towards next-generation biosensing. Trends Biotechnol. 2019;37(7):730–43.
30. Li SY, Cheng QX, Liu JK, Nie XQ, Zhao GP, Wang J. CRISPR-Cas12a has both cis- and trans-cleavage activities on single-stranded DNA. Cell Res. 2018;28(4):491–3.
31. Chen JS, Ma E, Harrington LB, Da Costa M, Tian X, Palefsky JM, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science. 2018;360(6387):436–9.
32. Zhou Q, Rahimian A, Son K, Shin DS, Patel T, Revzin A. Development of an aptasensor for electrochemical detection of exosomes. Methods. 2016;97:88–93.
33. Koh YQ, Almughlliq FB, Vaswani K, Peiris HN, Mitchell MD. Exosome enrichment by ultracentrifugation and size exclusion chromatography. Front Biosci (Landmark Ed). 2018;23:865–74.
34. Helwa I, Cai J, Drewry MD, Zimmerman A, Dinkins MB, Khaled ML, et al. A comparative study of serum exosome isolation using differential ultracentrifugation and three commercial reagents. PLoS One. 2017;12(1):e0170628.

35. Shpacovitch V, Hergenroder R. Optical and surface plasmonic approaches to characterize extracellular vesicles. a review. Anal Chim Acta. 2018;1005:1–15.
36. Greening DW, Xu R, Ji H, Tauro BJ, Simpson RJ. A protocol for exosome isolation and characterization: evaluation of ultracentrifugation, density-gradient separation, and immunoaffinity capture methods. Methods Mol Biol. 2015;1295:179–209.
37. Cheng N, Song Y, Shi Q, Du D, Liu D, Luo Y, et al. Au@Pd nanopopcorn- and aptamer nanoflower-assisted lateral flow strip for thermal detection of exosomes. Anal Chem. 2019;91(21): 13986–93.
38. Zhang P, Zhou X, He M, Shang Y, Tetlow AL, Godwin AK, et al. Ultrasensitive detection of circulating exosomes with a 3D-nanopatterned microfluidic chip. Nat Biomed Eng. 2019;3(6): 438–51.
39. Fu Z, Lu YC, Lai JJ. Recent advances in biosensors for nucleic acid and exosome detection. Chonnam Med J. 2019;55(2):86–98.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.