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Supplementary Information

Table S1 Details of the DNA sequences

Oligo Name	Sequence
H1 probe	TCAACATCAGTCTGATAAGCTATTATGTGTAGATAGC**TTC**TCAGACT
H1 probe	TCAACATCAGTCTGATAAGCTATTATGTGTAGATAGC**GGC**TCAGACT
H1 probe	TCAACATCAGTCTGATAAGCTATTATGTGTAGATAGC**TAC**TCAGACT
H1 probe	TCAACATCAGTCTGATAAGCTATTATGTGTAGATAGC**ATC**TCAGACT
H1 probe	TCAACATCAGTCTGATAAGCTATTATGTGTAGATAGC**CCC**TCAGACT
H1 probe	TCAACATCAGTCTGATAAGCTATTATGTGTAGATAGC**AAC**TCAGACT
H2 probe	TAAGCTATCTACACATGGTAGCTTATCAGACTCCATGTGTAGA
miR-21	TAGCTTATCAGACTGATGTTGA
miR-211	UUCCCUUUGUCAUCCUUCGCCU
miR-155	UUAAUGCUAAUCGUGAUAGGGGU
Let-7a	AGAGGTAGTAGGTTGCATAGTT
sgRNA	UAAUUUCUACUAAGUGUAGAUUAUAUAUACACCCCA
Reporter	BHQ1-CTGAGTGTGTGAGTTGCCCA- Cy3
U6-R	GCTAATCTTCTCTGTATCGTTCC
U6-F	GGTCGGGCAGGAAAGAGGGC

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Experimental section

1 Reagents and apparatus

HPLC-purified synthesized nucleic acids sequences were purchased from Sango Biotechnology Co., Ltd. (Shanghai, China). All DNA oligonucleotides were synthesized by Sangon Biotech Co. (Shanghai, China), and details of the synthesized sequences were illustrated in **Table S1**. Human breast

cancer cell line HeLa was purchased from China Center for Type Culture Collection in Shanghai. The patient's serum sample was taken from Southwest hospital and has passed ethical review.

2 Cell culture and exosomes extraction

Hela cells were cultured in medium containing RPMI-1640 with 10% FBS. The medium was then maintained in a humidified atmosphere of 5% CO_2 at 37°C. we then washed the cells with PBS solution for three times and collected the medium. The collected medium was centrifuged at 300 × g for 10 min to remove pellet and cells. Next, the supernatant was centrifuged at 1,000 × g to remove apoptotic bodies and debris. Then, the supernatant was centrifuged at 10,000 × g to remove large extracellular vesicles. The media supernatant was passed through a 0.22 µm pore filter (Millipore). The supernatant was subjected to ultracentrifgation at 100,000 × g for 2 h to sedment exosomes. The crude EVs were resuspended in pre-cooled PBS and mixed with ice-cold iodixanol/PBS to make a final 30% iodixanol solution. The resuspension was spread on the prepared density gradient (iodixanol solution concentration with 40%, 20%, 10%, and 5%, respectively. That is, the corresponding density gradients were 1.160, 1.147, 1.133, and 1.120g·mL^{-1}, Layered into the centrifuge tube from bottom to top in order). Ultra-concentration was performed at 4 °C for 16 hours with ultracentrifgation at 100,000 × g. The relevant fractions were collected, centrifuged at 100,000 × g for 1 h for precipitation and exosomes. The pellet was resuspended in 1.2 mL of PBS. Eventually, we characterized the size distribution and exosomal proteins of the obtained exosomes through NTA, transmission electron microscopy (TEM), and western blotting analysis, respectively.

3 Feasibility study of CHA and CRISPR-Cas9

The synthesized H1 and H2 probes were heated to 95 ℃ for 10 minutes, and then gradually lowered to room temperature within 2 hours. 1µl H1 probe, 1µl H2 probe and 1µl miRNA were mixed and adjusted to 10ul by DEPC water. After reacting for 1 hour at 37 ° C, the H1, H2, H1 + H2, H1 + H2 + miRNA was used loaded in 1% gel electrophoresis. In addition, the electrophoresis results in this experiment are the original real one directly from the SYSTEM Bio-Rad GelDoc XR$^+$ (Beijing, China), without post-processing.

To evaluate the sensitivity of the constructed detection method, the synthesized miRNA-21 was diluted to different concentrations (1nM, 100pM, 10pM, 1pM, 100fM), and the fluorescence signal of the system was detected by a fluorescence spectrophotometer, Hitachi FL700 (Beijing, China). Moreover, nucleic acid sequences such as miRNA211, miRNA155 and let-7a will be taken as interference, and the specificity of this method for miRNA21 has been evaluated.

In order to study the cleavage effect of Cas9 enzyme on dsDNA, we synthesized H1-H2 complex. Notably, the H2 were labeled with Cy3 and BHQ-1. We then mixed the 1µl H1-H2 complex (10mM)

with 1μl sgRNA (10mM) and 1ul (100ug / ml) Cas9 protein, and then detected their fluorescence intensity after incubating at 37 ° C for 30 min.

4 HeLa cell fixation and probe import

Select HeLa cells in the logarithmic growth phase to better maintain the morphological structure of the cells and tissues. After culturing the cells in the culture plate, aspirate the medium. Generally, the cells are gently washed with PBS for 2 × 3 min, and then fixed (apoptotic TUNEL staining can be left unwashed). Then, add 4% paraformaldehyde (prepared in PBS) for 20 min. If the histochemical test cannot be performed immediately, soak the cells with 0.4% paraformaldehyde in PBS PH7.4 (note that the cells should not be allowed to dry out before the immunohistochemical test is completed, otherwise the cell contraction shape is not good). The paraformaldehyde was removed and the cells were gently washed with PBS for 3 × 3 min. The method related probe and DAPI were then introduced into the fixed HELA cells, and the reaction was placed under confocal conditions after 30 minutes of reaction and PBS washing for 3 times.

5 Clinical application of the proposed method

We firstly collected blood samples of non-small cell lung cancer patients and healthy individuals from The Third Affiliated Hospital of Jinzhou Medical University. Plasma was obtained from blood samples after centrifugation at 5000 g for 10 min and filtered by a 0.22um filter. Then exosomes in each sample were collected through ultracentrifugation. The extracted exosomes were latterly lysed through exosomes lysate (20mM Tris-HCL, pH 7.4,1% Triton-X-100, 5mM EDTA, 150 mM NaCl, 2.5mM sodium pyrophosphate, 50mM sodium fluoride, 1 mM sodium orthovanadate, 1 mMβ-glycerophosphate). rt-qPCR and the proposed method were used to detect miRNA. Before the PCR method, miRNA was extracted from exosomes lysate: 200 μl of chloroform was added in the lysate and shake vigorously for 15 s, followed by centrifuge at 13,000 rpm for 10 minutes. We then carefully take the supernatant (about 600 μl) and transfer it to a new centrifuge tube. 900 μl ethanol were latterly mixed. After the mixture were centrifuged at 12,000 rpm for 30-60 seconds and washed for 3 times.