Supplementary Information

Rapid and sensitive detection of dual lung cancer-associated miRNA biomarkers by a novel SERS-LFA strip coupling with catalytic hairpin assembly signal amplification

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1. Results and discussion

1.1. Uniformity of the SERS-LFA strip

Fig. S1 showed the SERS mapping images acquired in the test zones (T1 and T2 lines) with the SERS intensities at 1083 cm^{-1} and 1330 cm^{-1} for different concentrations of target miR-196a-5p and miR-31-5p in PBS buffer and human serum, respectively. The scan area was 40×50 mm^2, the step size was 2 mm, the laser power was 5 mW, and the acquisition time at each point was 10 s. The color of SERS mapping was used to display the intensity of the 1083 cm^{-1} and 1330 cm^{-1} at each point according to a color scheme ranging from blue (lowest intensity) through green, yellow, orange, and red (highest intensity). With the miR-196a-5p concentration in PBS increased from 10 nM to 10 mM, although more SERS tags immunocomplexes were present in the T1 line, the color of SERS mapping was basically the same, which indicated that the SERS-LFA strip had a very uniform SERS enhancement effect. And the SERS mapping image always had uniform color distribution when the miR-31-5p concentration in PBS was increased from 10 nM to 10 mM. When the color distributions of the test lines in PBS (Fig. S1A and S1B) and in human serum (Fig. S1C and S1D) were compared, the mapping colors were also homogeneously distributed for all the concentrations of miR-196a-5p and miR-31-5p in human serum. The above results further confirmed high reproducibility and reliability of our method.

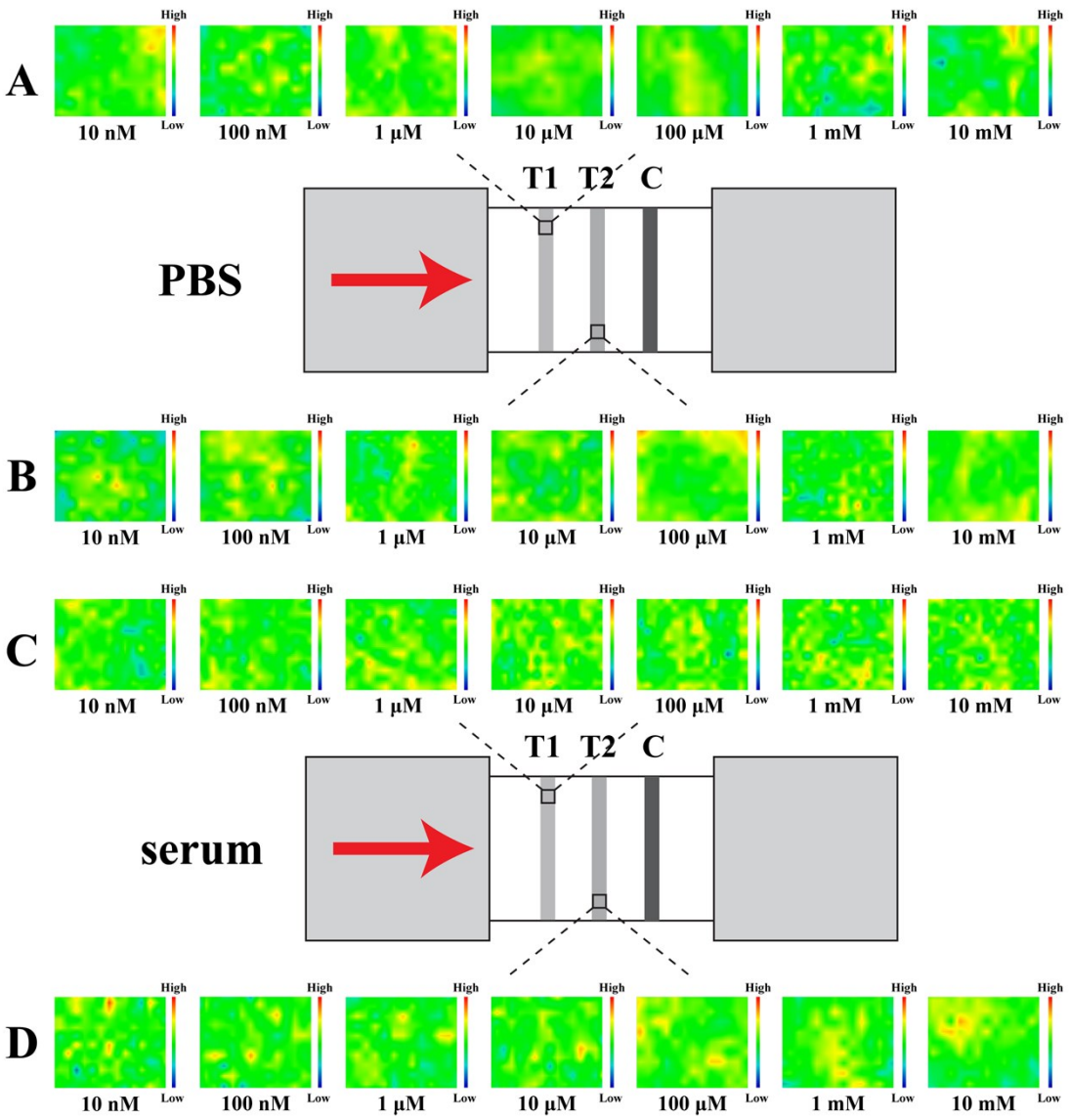

Fig. S1. (A) The corresponding SERS mapping of 4-ATP measured at 1083 cm^{-1} of T1 line on the SERS-LFA strip for different concentrations of target miR-196a-5p in PBS buffer. (B) The corresponding SERS mapping of DTNB measured at 1330 cm^{-1} of T2 line on the SERS-LFA strip for different concentrations of target miR-31-5p in PBS buffer. (C) The corresponding SERS mapping of 4-ATP measured at 1083 cm^{-1} of T1 line on the SERS-LFA strip for different concentrations of target miR-196a-5p in human serum. (D) The corresponding SERS mapping of DTNB measured at 1330 cm^{-1} of T2 line on the SERS-LFA strip for different concentrations of target miR-31-5p in human serum.

1.2. Comparison of different methods used for miRNAs detection

Table S1 was comparison of this work with other miRNAs detection methods. Generally, the LOD of the proposed method was lower than most reported methods (Table S1), indicating that the proposed strategy was more sensitive than the reported techniques.

Table S1. Comparison of this work with other miRNAs detection methods.

Method	Analyte	Limit of detection (LOD)	Ref.
Fluorescence Colorimetry	miR-150-5p	9.05 nM	1
Colorimetry	BKV DNA	5 nM	2
Colorimetry	Pseudorabies virus	200 copies	3
Structurally Responsive Assay	miRNA-21	0.115 nM	4
SERS	miR-196a-5p	1.681 nM	This work
	miR-31-5p	2.603 nM	

1.3. Feasibility evaluation of CHA

In order to testify the feasibility of the design, gel electrophoresis was conducted to monitor the happen of CHA between H1 and H2 by using the target miR-196a-5p and miR-31-5p as an initiator. In Fig. S2, when H1 and H2 were mixed together (lane 5), almost all H1 and H2 appeared banded as they were separately loaded in lane 2 and lane 3 respectively. Almost no H1-H2 hybridization products formed at the top of lane 5, which proved that no CHA happened between H1 and H2 in the absence of target miR-196a-5p and miR-31-5p. When H1 was incubated with the target miRNAs (T), the hairpin structure of H1 could be opened to form the H1-T complex, which appeared in lane 6. Further addition of H2 to the H1-T complex resulted in the appearance of a strong band of H1-H2 complex and release of T (lane 7). Such results

obviously proved the happen of CHA.

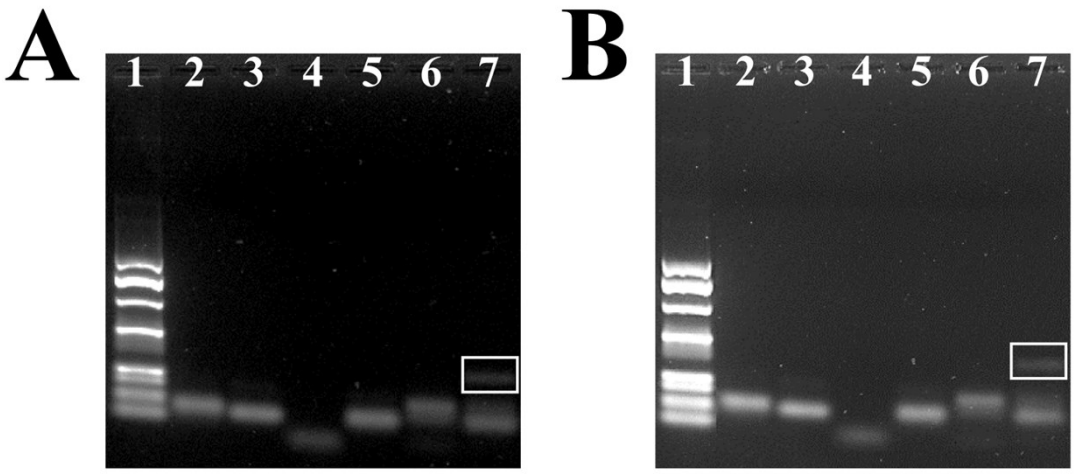

Fig. S2. Gel electrophoresis for DNA and RNA stands. A: lane 1: Marker; lane 2: H1-1; lane 3: H2-1; lane 4: T (miR-196a-5p); lane 5: H1-1 + H2-1; lane 6: H1-1 + T; lane 7: H1-1 + H2-1 + T. B: lane 1: Marker; lane 2: H1-2; lane 3: H2-2; lane 4: T (miR-31-5p); lane 5: H1-2 + H2-2; lane 6: H1-2 + T; lane 7: H1-2 + H2-2 + T.

References

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