Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

MicroRNA-activated molecular machine for non-enzymatic target recycling amplification detection of microRNA from cancer cells

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Experimental Section

Reagents and Materials: All oligonucleotides listed in Table S1 were synthesized and purified by Shanghai Sangon Biological Engineering Technology and Services (Shanghai, China). Other reagents were obtained from Kelong Chemical Inc. (Chengdu, China). All reagents as received were of analytical grade and used without further purification, and solutions in the experiments were prepared with ultrapure water (specific resistance of 18.3 MΩ•cm).

Table S1 Sequences of the oligonucleotides used in the experiments.

Name	Sequence
Template strand (TS)	5'-CCA TCT TTA CCA GAC AGT GTT AGG GAT(DABCYL) CGT AAG TTA GTT GGA GAC GTA GG-3'
Assistant Probe (AP)	5'-CTT TCC TAC ACC TAC GTC TCC AAC TAA CTT ACG A(DABCYL)-3'
Signal Probe (SP)	5'-CCA CAT ACA TCA TAT TT(FAM)C CCT AAC ACT GTC TGG TAA-3'

Fuel Strand (FS)	5’-CC TAC GTC TCC AAC TAA CTT ACG ATC CCT AAC ACT GTC TGG TAA-3’
miR-141	5’-UAACACUGUCUGGUAAAGAUGG-3’
miR-200a	5’-UAACACUGUCUGGUAACGAUGU-3’
miR-200b	5’-UAAUACUGCCUGGUAAUGAUGA-3’
miR-21	5’-UAGCUUAUCAGACUGAUGUUGA-3’

Cell culture and preparation of cellular extracts: The two human cancer cell lines, the human prostate (22Rvl) and human cervical (HeLa) cancer cell lines, were purchased from the Cell bank of type culture collection of the Chinese Academy of Science (Shanghai, China). 22Rvl cells and HeLa cells were grown in glass-bottom dishes in a humidified atmosphere at 37 ºC containing 5% CO_2, using RPMI 1640 medium supplemented with 10% fetal bovine serum and 100U mL^{-1} penicilin-streptomycin. The cells were then collected and re-suspended after trypsinization and washing with fresh medium. The cellular extracts were prepared by using the Trizol Reagent Kit from Life Technologies (Shanghai, China) according to the manufacture’s protocol. Briefly, cells were mixed and lysed with an appropriate amount of Trizol Reagent, followed by the addition of chloroform and centrifugation. The RNA extracts in the aqueous phase were precipitated with isopropyl alcohol and transferred to a 200 μL microcentrifuge tube. Finally, the extracted RNA solution was diluted and stored at -20 ºC for further use.

MiRNA-141 sensing protocol: The three-strand DNA complex probes were prepared by annealing the mixture of the TS (200 nM), AP (200 nM) and SP (200 nM) in Tris-buffer at 90 °C for 5 min, followed by cooling the mixture down to room temperature. The FS was then added to the mixture to prepare the sensing solution. For target miRNA

detection, miRNA-141 at different concentrations were added to the sensing solutions and incubated for 75 min at room temperature, followed by fluorescence measurements.

Fluorescence measurements: Fluorescence measurements were performed on a RF-5301PC spectrophotometer (Shimadzu, Tokyo, Japan) utilizing excitation and emission slits of 3 and 5 nm with a 150W Xenon lamp (Ushio Inc, Japan) as the excitation source at room temperature. The emission spectra were collected from 450 nm to 580 nm with excitation wavelength of 490 nm, and the fluorescence intensity was measured at 520 nm.

Supplementary Figure:

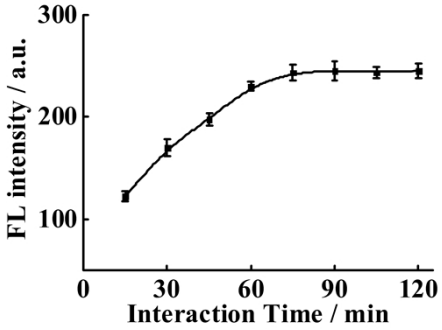

Fig. S1 Effect of reaction time on the fluorescence intensity of the assay method. The concentrations of the DNA complex probes, FS and miRNA-141 are 200 nM, 200 nM and 1 nM, respectively.

Our method for miRNA-141 detection is based on homogeneous target recycling amplification, and the reaction time plays a crucial role on the analytical performance of the method. In order to obtain optimal condition for this assay, the reaction time was investigated by incubating the miRNA-141 (1 nM) with the probe solution containing the DNA complex probes (200 nM) and FS (200 nM) from 0 to 120 min at a time interval of 15 min. From Fig. S1, we can see that as the reaction time is prolonged, the fluorescence

intensity increases gradually from 0 to 75 min and almost levels off thereafter. Based on this optimization, the reaction time of 75 min is used for subsequent experiments.