Electronic Supplementary Material (ESI) for ChemComm.
This journal is © The Royal Society of Chemistry 2018

Method to directly assay circRNA in real sample

Jin Jiao,[a] Tao Gao,[a] Hai Shi,[a] Anzhi Sheng,[a] Yang Xiang,[*a] Yongqian Shu[*c] and Genxi Li*[ab]

[a] State Key Laboratory of Pharmaceutical Biotechnology and Collaborative Innovation Center of Chemistry for Life Sciences, Department of Biochemistry, Nanjing University, Nanjing 210093, P. R. China
[b] Center for Molecular Recognition and Biosensing, School of Life Sciences, Shanghai University, Shanghai, 200444, P. R. China
[c] Department of Oncology, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, P. R. China

1 Experimenal section

1.1 Materials

The fluorophore/quencher-labeled molecular beacons were synthesized and purified by Sangon Inc. (Shanghai, China). Duplex-specific nuclease (DSN) was obtained from Evrogen Joint Stock Company (Russia). RNase inhibitor was purchased from Fermentas Inc. (Vilnius, Lithuania). 1 unit/μL RNase R was obtained from Epicenter (Madison, WI, USA). All chemicals were purchased from Sigma-Aldrich, Inc. (Saint Louis, MO, USA) and used without further purification. In order to create and maintain an RNase-free environment, all reagents used in this method were dissolved with water-DEPC treat, and the tips and tubes are RNase-free. One Step PrimeScript® miRNA cDNA Synthesis kit and SYBR®Premix Ex TaqTM II kit were purchased from Takara Biotechnology Co. Ltd. (Dalian, China). The T7 RNA polymerase that transcribes DNA into RNA and the T4 RNA ligase that circularisationof linear RNA are obtained from Takara. The buffers for cell experiments were prepared using distilled water purified by a Milli-Q water purification system (Millipore Corp., Bedford, MA). Total RNA was extracted from each specimen using Trizol reagent (Sangon Inc.) according to the manufacturer's instruction. RNA concentration was determined by ScanDrop Nuclear Acid Analyzer (analytikjena, German). Related sequences such as molecular beacons and primers are listed in Table S1.

1.2 Instrumentation.

The fluorescence emission spectra were recorded by a fluorescence microplate reader (BioTek Instrument, Winooski, VT, USA) with different excitation wavelength according to fluorophore using a black 384 well microplate (Fluotrac 200, Greiner, Germany). The measurement settings were λex= 485 nm for FAM dye. The real-time PCR experiments were conducted in Steponeplus (Applied Biosystems, CA, USA).

1.3 Synthesis and Circularisation of linear RNA.

The circular RNA was prepared according to the previous reported method with minor modification. Briefly, linear RNAs were transcribed in vitro by T7 RNA polymerase from corresponding dsDNA templates. The dsDNA templates were synthesised in Sangon Inc. The reaction was treated with DNase to remove the DNA template. Transcribed linear RNAs were annealed to its guide DNA and then ligated using T4 RNA ligase 2 to produce the circular RNA. Circularity check of the RNA using RNase R. RNA was incubated with RNase R (1 unit/μL) in 20 mM Tris-HCl (pH 8.0), 0.1 M KCl, and 0.1 mM $MgCl_2$ at 37 °C for 10 min. The reaction mixture (10 μL) was analyzed by 2% denaturing agarose gel electrophoresis.

1.4 DSN Method for circRNAs Detection.

First, a volume of 25 μL reaction mixture containing 1×DSN buffer, 0.08 U DSN (dissolved in 25mM Tris-HCl, pH 8.0; 50% glycerol), 20 U RNase inhibitor, molecular beacons and miRNA target, after add 1 drop of paraffin oil to each sample incubated the solution in a thermal cycler for 25 min. According to the previous reported, we set 60℃ as the reaction temperature.[1] Subsequently, the reaction mixture was added 45 μL inhibitor (10 mM EDTA) and incubated at 60 °C for 5 min to inactive DSN enzyme. Following that, the reaction mixture was transferred to a black 384 well microtiter plate to measure fluorescence signal. Quantification of the circRNA is carried out by fluorescence emission spectra were recorded.

1.5 Cell Culture and Preparation of Lysates.

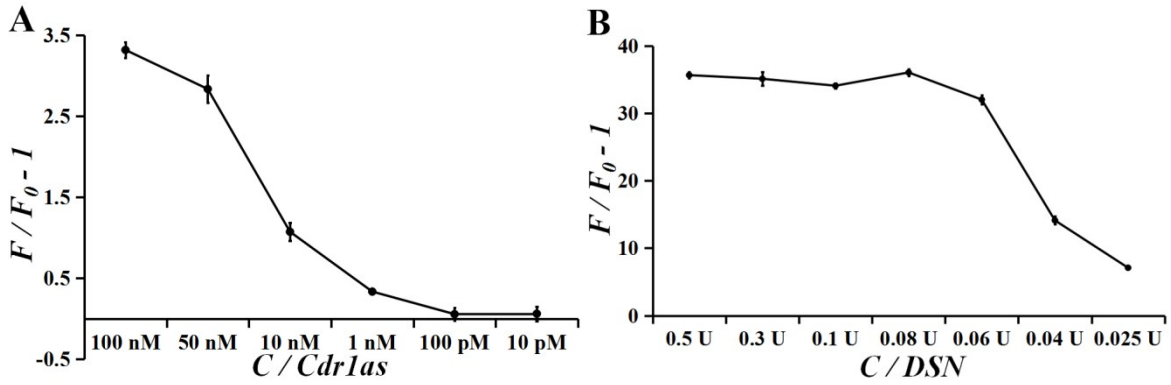
All appliances including the centrifuge tubes, spearheads, and culture dishes, were dried in an oven after a sterilization process. The solutions used in all experiments were prepared using water-DEPC treated. Human colorectal cancer cell line (SW480), human breast cancer cells (MCF-7), human liver cancer cell lines (HepG2) and human bronchial normal cell lines (HBE) were obtained from the cell bank of the type culture collection of the state key laboratory of pharmaceutical biotechnology (Nanjing, China). SW480 cells were cultured in RPMI 1640 medium (Thermo Scientific Hyclone) supplemented with 10% fetal bovine serum, 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin at 37°C in a humidified 5% CO_2 incubator. MCF-7, HepG2 and HBE cells were respectively cultured in Dulbecco's modified Eagle medium (DMEM). The cellular lysates were prepared according to the reported method, by using for experiments after RNase R treatment.

Table S1. Oligonucleotide sequences designed in this study.

Name	Sequence (5'-3')
Adsorption site	GUCUUCCAGAAAAUC
Forward primer	TCAACTGGCTCAATATCCATGTC
Reverse primer	ACCTTGACACAGGTGCCAT
Guide DNA	TGCAATATCCAGGGTTTCCGATGGC
DNA probes	FAM-CCGCGCGATTTTCTGGAAGACGCGCGG-BHQ
1 M[a]	FAM-CCGCGCGAATTTCTGGAAGACGCGCGG-BHQ
2 M	FAM-CCGCGCGAATTTCTGGAATACGCGCGG-BHQ
3 M	FAM-CCGCGCGAATTTCTCGAATACGCGCGG-BHQ
4 M	FAM-CCGCGCGAATTTCTCGAATGCGCGCGG-BHQ

[a]For DNA probes, the mutant ones are abbreviated as M (1 - 4M), respectively.

Figure S1. Fluorescence ratio values of (F/F_0 - 1) upon the control group and different amounts of duplex-specific nuclease (DSN) enzyme.



In this part, a control group without DSN could detect that aCdr1as at a concentration of 1 - 100nM has been confirmed as shown in Figure S1A. In addition, according to the fluorescence ratio values of (F/F_0 - 1) upon the different amounts of DSN enzyme with 100nM aCdr1as and 1 μM DNA probes, the optimal DSN amount was selected as 0.08 units (U) (Figure S1B). Fluorescence emission spectra excitation at 485 nm, emission at

518 nm; where F_0 and F are the fluorescence signals in the absence and the presence of aCdr1as; error bars, SD, n = 3.

Reference

1. B. C. Yin, Y. Q. Liu and B. C. Ye, Journal of the American Chemical Society, 2012, **134**, 5064-5067.