Supporting Information

Conditional Regulation of HCR for Rapid Visualization of Endogenous miR-21 in Cancer Cells

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CONTENT

1. Materials and methods

1.1 Reagents and materials

The oligonucleotide was chemically synthesized by General Biosystems Co., Ltd (Anhui, China). Acrylamide, Acryl/Bis 30% Solution (29:1), Water-DEPC Treated Water, RNase-free ddH_2O, Ethidium bromide bought by Sangon Biotech Co., Ltd (Shanghai, China). Dulbecco's modified essential media (DMEM), Hyclone fetal bovine serum (FBS), Hoechst 33342 (62249), AF568-NHS Ester, Polyethylenimine, Lipofectamine™ 3000 Transfection Reagent were purchased from ThermoFisher Scientific. Hela cells (ATCC): CCL-2, human, female; HEK293T(ATCC): CRL3216, human, sex unknown; 3T3(ATCC): L1, mouse, sex unknown. All other chemicals, including solvents, were purchased from Sigma-Aldrich, Aladdin, and Adamas and used without further purification.

1.2 Oligonucleotide labeling

The oligonucleotide was synthesized and purified by PAGE. FAM or active amino group were modified on oligonucleotide chains H_1 and H_2, respectively. The oligonucleotide was dissolved in ddH_2O and used directly for transcription in vitro. The purified H_2 oligonucleotide and AF568-NHS Ester (molar ratio of 3:1) were incubated in PBS buffer (20 mM, pH 7.4) at room temperature for 2 h and then purified by G-25 prepacked column. The modified H_1-FAM and H_2-AF568 oligonucleotide chains were dissolved in the hairpin folding solution (20 mM Tris-HCl, 137 mM NaCl, pH 7.4) to produce a mother solution with a final concentration of 10 μM.

1.3 Nucleic acids PAGE electrophoresis

All appliances were treated with ddH_2O water to remove bacteria and RNase. Each 10 mL contains 6.66 mL 30% acrylamide, 1 mL 50×TAE, 50 μL 10% AP, and 5 μL TEMED, the final concentration of electrophoresis gel was 15%. In vitro experiments, the uracil in the RNA

sequence of miR-21 was replaced with thymine called "I" (Table S1) to become a single-stranded DNA (ssDNA) sequence to resist RNA degradation, which did not affect the complementary base pairing between nucleic acid chains. The initiator chain "I" and the folded hairpin chains "H_1" and "H_2" are mixed at different concentrations and react at room temperature for 4 h or 37 ℃ 2 h. Then the 9 μL reacted sample is mixed with 1 μL 10×loading buffer and added directly into the polyacrylamide gel without heating. After 135 V for 50 min, it was stained with EB in ddH_2O for 10 min. The gel is photographed under ultraviolet light. The extraction of image signal is realized through ImageJ.

1.4 HCR reaction under different buffers

After the oligonucleotide chains "H_1" and "H_2" were folded in the nucleic acids hairpin folding solution, they were added to six different assembly buffers respectively with the simulated nucleic acid chain "I" of miR-21, and reacted at 37 ℃ 2 h to varify the influence of different ion concentrations on the HCR assembly reaction. The six different buffer components are as follows: buffer 1, 20 mM PBS, 10 mM $MgCl_2$; buffer 2, 20 mM PBS, 5 mM $MgCl_2$, 50 mM NaCl; buffer 3, 20 mM PBS, 10 mM $MgCl_2$, 50 mM NaCl; buffer 4, 20 mM PBS, 5 mM $MgCl_2$, 50 mM NaCl, 20 mM HEPES; buffer 5, 20 mM PBS, 10 mM $MgCl_2$, 50 mM NaCl, 20 mM HEPES; buffer 6, 20 mM PBS, 5 mM $MgCl_2$. Then after polyacrylamide gel electrophoresis, the gel is photographed under ultraviolet light. The extraction of image signal is realized through ImageJ.

1.5 Live-cell fluorescence imaging

Hela, 293T, 3T3 cells were seeded into a confocal imaging plate with 70-80% confluency. The cells were transfected with 300 ng H_1-FAM and 300 ng H_2-AF568 in sequence per plate (200 μL) using Lipofectamine 3000 (2 μL) and incubated for 2h. The cells were then incubated with the fresh medium containing 500 nM Hoechst 33342. After incubation at 37 ℃ for 1 h with 5% CO_2, the fluorescence imaging was performed with a 100 × oil-dripping objective lens by using an ANDORTM living cell laser scanning confocal microscope (Revolution WD). For H_1-FAM: λ_{ex} = 488 nm, λ_{em} = 500-570 nm, For H_2-AF549, λ_{ex} = 561 nm, λ_{em} = 580-650 nm.

The extraction of image signal is realized through ImageJ.

2. Supplementary Figures and Tables

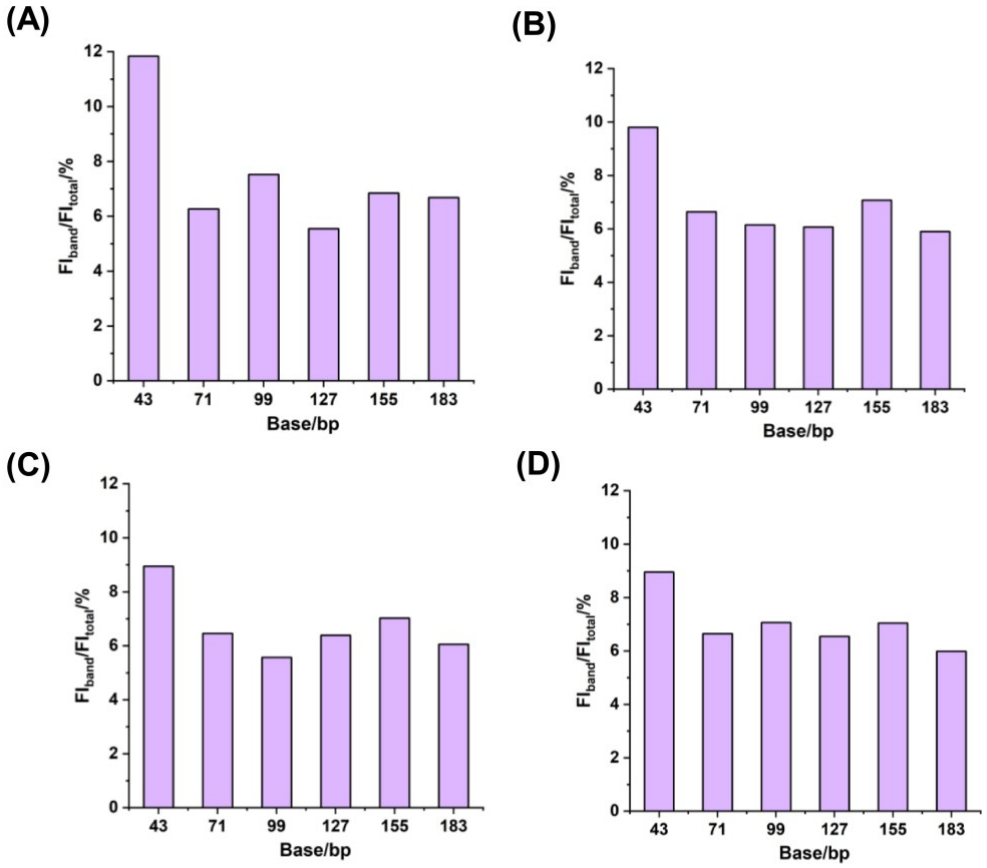

Fig. S1(A) - (D) The proportion of different types of nucleic acid assemblies, and the concentration of miR-21 is 1 μM, 0.8 μM, 0.5 μM, 0.3 μM, respectively.

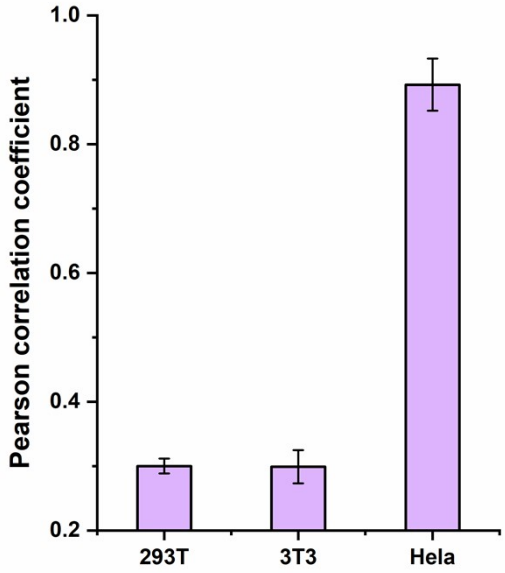

Fig. S2 Colocalization of H1-FAM and H2-AF568 in three different cell lines in Figure 6A-C;

Table S1 The oligonucleotide sequences of HCR.

Name	Sequence 5'-3'
miR-21	UAGCUUAUCAGACUGAUGUUGA
I	TAGCTTATCAGACTGATGTTGA
H_1	TCAACATCAGTCTGATAAGCTACTAAGTTAGCTTATCAGACTG
H_2	TAGCTTATCAGACTGATGTTGACAGTCTGATAAGCTAACTTAG
H_1-FAM	TCAACATCAGTCTGATAAGCTACTAAGTTAGCTTATCAGACTG-FAM
H_2-AF568	AF568-TAGCTTATCAGACTGATGTTGACAGTCTGATAAGCTAACTTAG