Labeling of target mRNAs by a photo-reactive microRNA probe

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Supplementary Data

RNA Synthesis. Synthesis was carried out with a DNA/RNA synthesizer by phosphoramidite method. Deprotection of bases and phosphates was performed in concentrated NH_4OH:EtOH (3:1, v/v) at 55 ºC for 4 h. 2′-TBDMS groups were removed by TEA·3HF (Aldrich) at 65 ºC for 1.5 h. The reaction was quenched with 0.1 M TEAA buffer (pH 7.0) and desalted on a Sep-Pak C18 cartridge. Deprotected ONs were purified by 20% PAGE containing 7 M urea to give the highly purified RNA1 (9), RNA2 (6), RNA3 (5), RNA4 (5), RNA5 (5), RNA6 (3), RNA7 (5), RNA8 (4), RNA9 (4), RNA10 (4), RNA11 (5), RNA12 (4), RNA13 (5), RNA14 (4), RNA15 (7), RNA16 (4), RNA17 (5), RNA18 (4), RNA19 (6), RNA20 (5), RNA21 (7), RNA22 (4), RNA23 (4), RNA24 (4), RNA25 (4), RNA26 (4). The yields are indicated in parentheses as OD units at 260 nm starting from 0.2 μmol scale.

MALDI-TOF/MS Analysis of RNAs. Spectra were obtained with a SHIMAZU/KRATOS time-of-flight mass spectrometer equipped with a nitrogen laser (337 nm, 3-ns pulse). A solution of 3-hydroxypicolinic acid (3-HPA) and diammonium hydrogen citrate in H_2O was used as the matrix. RNA1: *m/z* = 6979.8 ($[M–H]^–$, calculated 6979.2; $C_{208}H_{257}N_{78}O_{155}P_{21}$ (MW = 6980.2)). RNA2: *m/z* = 7220.3 ($[M–H]^–$, calculated 7219.3; $C_{215}H_{270}N_{78}O_{162}P_{22}$

(MW = 7220.3)). RNA3: *m/z* = 7789.8 ([M–H]$^-$, calculated 7788.9; $C_{237}H_{310}N_{81}O_{172}P_{23}$ (MW = 7789.9)). RNA4: *m/z* = 7855.9 ([M–H]$^-$, calculated 7854.0; $C_{241}H_{312}F_3N_{78}O_{172}P_{23}$ (MW = 7855.0)). RNA5: *m/z* = 7892.1 ([M–H]$^-$, calculated 7893.0; $C_{242}H_{313}F_3N_{81}O_{171}P_{23}$ (MW = 7894.0)). RNA6: *m/z* = 7894.3 ([M–H]$^-$, calculated 7894.0; $C_{242}H_{312}F_3N_{80}O_{171}P_{23}$ (MW = 7895.0)). RNA7: *m/z* = 7893.1 ([M–H]$^-$, calculated 7894.0; $C_{242}H_{312}F_3N_{80}O_{171}P_{23}$ (MW = 7895.0)). RNA8: *m/z* = 7870.1 ([M–H]$^-$, calculated 7869.9; $C_{241}H_{312}F_3N_{78}O_{173}P_{23}$ (MW = 7871.0)). RNA9: *m/z* = 7854.5 ([M–H]$^-$, calculated 7854.0; $C_{241}H_{312}F_3N_{78}O_{172}P_{23}$ (MW = 7855.0)). RNA10: *m/z* = 7893.2 ([M–H]$^-$, calculated 7893.0; $C_{242}H_{313}F_3N_{81}O_{171}P_{23}$ (MW = 7894.0)). RNA11: *m/z* = 7895.3 ([M–H]$^-$, calculated 7893.0; $C_{242}H_{313}F_3N_{81}O_{171}P_{23}$ (MW = 7894.0)). RNA12: *m/z* = 7893.7 ([M–H]$^-$, calculated 7893.0; $C_{242}H_{313}F_3N_{81}O_{171}P_{23}$ (MW = 7894.0)). RNA13: *m/z* = 7895.3 ([M–H]$^-$, calculated 7893.0; $C_{242}H_{313}F_3N_{81}O_{171}P_{23}$ (MW = 7894.0)). RNA14: *m/z* = 7893.8 ([M–H]$^-$, calculated 7894.0; $C_{242}H_{312}F_3N_{80}O_{171}P_{23}$ (MW = 7895.0)). RNA15: *m/z* = 7894.1 ([M–H]$^-$, calculated 7894.0; $C_{242}H_{312}F_3N_{80}O_{171}P_{23}$ (MW = 7895.0)). RNA16: *m/z* = 7895.4 ([M–H]$^-$, calculated 7894.0; $C_{242}H_{312}F_3N_{80}O_{171}P_{23}$ (MW = 7895.0)). RNA17: *m/z* = 7871.1 ([M–H]$^-$, calculated 7869.9; $C_{241}H_{312}F_3N_{78}O_{173}P_{23}$ (MW = 7871.0)). RNA18: *m/z* = 7854.6 ([M–H]$^-$, calculated 7854.0; $C_{241}H_{312}F_3N_{78}O_{172}P_{23}$ (MW = 7855.0)). RNA19: *m/z* = 7856.6 ([M–H]$^-$, calculated 7854.0; $C_{241}H_{312}F_3N_{78}O_{172}P_{23}$ (MW = 7855.0)). RNA20: *m/z* = 7876.7 ([M–H]$^-$, calculated 7869.9; $C_{241}H_{312}F_3N_{78}O_{173}P_{23}$ (MW = 7871.0)). RNA21: *m/z* = 7871.1 ([M–H]$^-$, calculated 7869.9; $C_{241}H_{312}F_3N_{78}O_{173}P_{23}$ (MW = 7871.0)). RNA22: *m/z* = 7894.3 ([M–H]$^-$, calculated 7893.0; $C_{242}H_{313}F_3N_{81}O_{171}P_{23}$ (MW = 7894.0)). RNA23: *m/z* = 7896.1 ([M–H]$^-$, calculated 7894.0; $C_{242}H_{312}F_3N_{80}O_{171}P_{23}$ (MW = 7895.0)). RNA24: *m/z* = 7895.1 ([M–H]$^-$, calculated 7894.0; $C_{242}H_{312}F_3N_{80}O_{171}P_{23}$ (MW = 7895.0)). RNA25: *m/z* = 7894.4 ([M–H]$^-$, calculated 7894.0; $C_{242}H_{312}F_3N_{80}O_{171}P_{23}$ (MW = 7895.0)). RNA26: *m/z* = 7894.2 ([M–H]$^-$, calculated 7893.0; $C_{242}H_{313}F_3N_{81}O_{171}P_{23}$ (MW = 7894.0)).

Photocrosslinking. The RNA probes containing the analogue **1** or **2** were annealed with complementary RNAs, which were labeled with fluorescein at their 5′ end, in a buffer of 10 mM Tris-HCl (pH 7.2) and 0.1 M NaCl. The mixtures were irradiated with 365 nm UV-A for 30 min and then 302 nm UV-B for 10 min at 0 °C (~1 cm under 6 W UV-B or UV-A

bulb), and the photocrosslinked products were analyzed by 20% PAGE under denaturing conditions (Fig. S1). RNA probes used in this experiment are listed in Table S1.

Western blotting. DLD-1 cells were grown in RPMI-1640 medium supplemented with 10% (v/v) heat-inactivated FBS (Aldrich) and 2 mM L-glutamine under an atmosphere of 95% air and 5% CO_2 at 37 °C. 24 h before transfection, DLD-1 cells (0.5×10^5/mL) were transferred to a 6-well plate (1 mL/well). They were transfected using Lipofectamin RNAiMAX (Invitrogen), according to the manufacturer's protocol. After 48 h, whole cells were homogenized in chilled lysis buffer (10 mM Tris-HCl (pH 7.4), 1% NP-40, 0.1% deoxycholic acid, 0.1% SDS, 150 mM NaCl, 1 mM EDTA, and 1% Protease Inhibitor Cocktail) and stood for 20 min on ice. After centrifugation at 13,000 rpm for 20 min at 4 °C, the supernatants were collected as whole cell protein samples. Protein contents were measured with a DC Protein assay kit (Biorad, Hercules). 10 μg of lysate protein was separated by 12.5% SDS–PAGE, and blotted onto a PVDF membrane (PerkinElmer Life Sciences). After blockage of nonspecific binding sites, the membrane was incubated overnight at 4 °C with primary antibody followed by incubation with HRP-conjugated secondary antibody (Cell Signaling Technology) at room temperature. After washed the membrane, the immunoblots were visualized by use of Ammersham ECL Plus Western Blotting Detection Reagents (GE Healthcare) according to the manufacture's protocol.

Labeling of target mRNAs. 24 h before transfection, DLD-1 cells (3.0×10^6/mL) were transferred to a 6-well plate (1.5 mL/well). They were transfected with miRNA using Lipofectamin RNAiMAX. After 24h, solution in each well was removed and PBS buffer was added (1.5 ml/well). The cultured cells were irradiated with 365 nm UV-A for 15 min at 0 °C (~1 cm under 6 W UV-A bulb) and then, total RNA was isolated by using a NucleoSpin miRNA isolation kit (TaKaRa). To purify the target mRNAs, 18 μg of total RNA and 150 μL of Dynabeads M-280 Streptavidin (Invitrogen) were incubated at 4 °C for 1h. After the supernatant was removed, dynabeads was washed three times with 500 μL of 0.1% NP-40. 50 μL of H_2O and 200 μL of TRIZOL reagent were added to dynabeads and released target mRNAs were collected by using a NucleoSpin miRNA isolation kit. For determination of the expression levels, each RNA samples were reverse-transcribed with a

SuperScript VILO cDNA Synthesis Kit (Invitrogen). Real-time PCR was performed with primers specific for target mRNA by using THUNDERBIRD SYBR qPCR Mix (TOYOBO). Primers used in this experiment are listed in Table S2.

Table S1. Primes used for determination of target mRNA levels.

Abbreviation of primer[a]	Sequence
FSCN1-F	5′-r(AATCAGGACGAGGAGACCGA)-3′
FSCN1-R	5′-r(ACAAACTTGCCATTGGACGC)-3′
KLF4-F	5′-r(GATGATGCTCACCCCACCTT)-3′
KLF4-R	5′-r(GCGAATTTCCATCCACAGCC)-3′
c-MYC-F	5′-r(CGTCCTCGGATTCTCTGCTC)-3′
c-MYC-R	5′-r(GCTGGTGCATTTTCGGTTGT)-3′
β-actin-F	5′-r(GAGCACAGAGCCTCGCCTTT)-3′
β-actin-R	5′-r(CCTCGTCGCCCACATAGGAA)-3′

[a]Fs denote forward primers. Rs denote reverse primers.

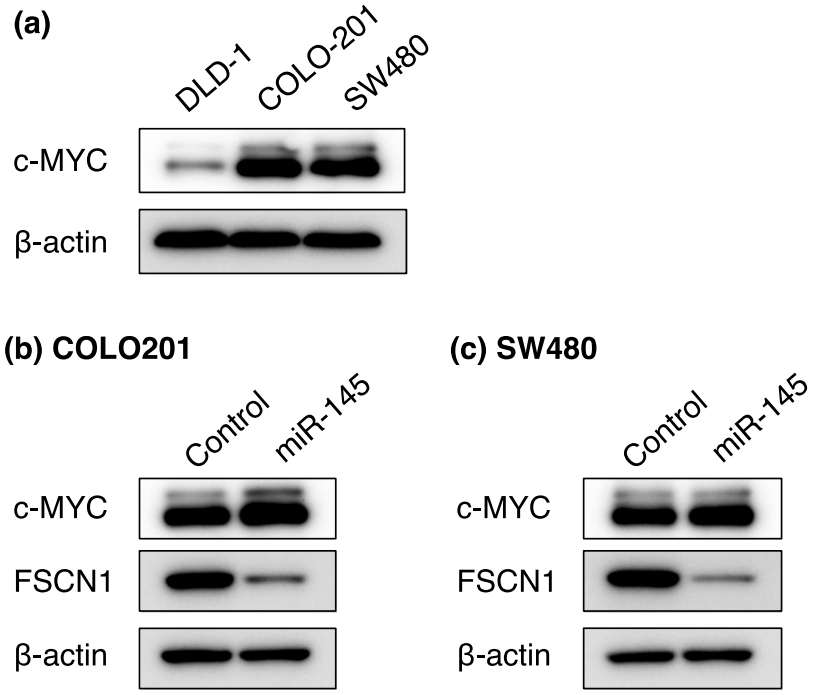

Figure S1. (a) Relative expression levels of *MYC* protein in the human colon cancer cells. (b) Gene silencing activities of the synthesized miR-145 probes in COLO201 cells. (c) Gene silencing activities of the synthesized miR-145 probes in SW480 cells.