Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Site-Specific Photochemical RNA Editing

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Experimental Section

General

Mass spectra were recorded on a Voyager-DE PRO-SF, Applied Biosystems. Irradiation was performed by UV-LED (OMRON, ZUV, 366 nm, 1,600 mW/cm^2) or 15 W transilluminator (FUNAKOSHI, TR-312R/J, 312 nm). HPLC was performed on a Chemcobond 5-ODS-H column (10 × 150 mm, 4.6 × 150 mm) or a Chemcosorb 5-ODS-H column (4.6 × 150 mm) with a JASCO PU-980, HG-980-31, DG-980-50 system equipped with a JASCO UV 970 detector at 260 nm. Reagents for the DNA synthesizer such as A, G, C, T-β-cyanoethyl phosphoramidite, and CPG support were purchased from Glen Research.

Preparation of ^{CNV}K-modified oligonucleotides

The phosphoramidite of ^{CNV}K was prepared according to a method reported in literature[1]. The modified oligonucleotides containing ^{CNV}K were prepared, according to standard phosphoramidite chemistry, on a DNA synthesizer using the phosphoramidite of ^{CNV}K as shown in Table 1. Oligonucleotides containing ^{CNV}K were purified by reverse-phase HPLC and characterized by MALDI–TOF–MS.

RNA editing

A buffer solution of RNA (1 μM) and ^{CNV}K-ODN (1.5 μM) (50 mM Na-cacodylate (pH 7.4), 100 mM NaCl, 33 μL) was UV-irradiated (366 nm, 30 sec, 0ºC) and the solution was heated (3.5 h, 90ºC). Then the solution was UV-irradiated (312 nm, 5 min, 80ºC).

Sequence analysis

In the case of model RNAs, RNA(G<u>C</u>G), RNA(C<u>C</u>G), RNA(G<u>C</u>C) and RNA(C<u>C</u>C), product after the photochmical RNA editing was reverse-transcribed by AMV-RT (Promega) with RT primer (RT-R; GTGGATCCTGGATATAGTTCCTCCTTTCAGCCCCGGGCCTGCAGGAT CGATGTTTTTTTTTTGT). Then obtained cDNA was amplified by PCR (PrimeSTAR (TaKaRa), 98ºC 5 sec, 52ºC 10 sec, 68ºC 5 sec, 30 cycles) with PCR primer (RT-R and RT-F; GTGGATCCTTAATACGACTCACTATAGGGCCCGGGCCTGCAGGATCGATGAA CCATACAC). After the purification of cDNA by QIA quick PCR purification kit (QIAGEN), the sequence of the amplified cDNA was analyzed by Sanger's method with the sequence primer (TTAATACGACTCACTATAGGG). In the case of miR-27b, cDNA of the product after the photochmical RNA editing was obtained by small RNA cloning kit (TaKaRa). The cDNA

was amplified by PCR (PrimeSTAR (TaKaRa), 98ºC 5 sec, 55ºC 10 sec, 68ºC 5 sec, 30 cycles) with PCR primer (mir-27F; AAAGTGGATCCTTAATACGACTCACTCTCGGGTACGACTCGGTGAAAGATCCTGC, mir-27R; AAAGGATCCAGTGAGTCGTATTAATTTCGCGGGAGTCTCTAGCC). After the purification of cDNA by agarose gel electrophoresis, the sequence of the amplified cDNA was analyzed by Sanger's method with the sequence primer (mir-27F or mir-27R).

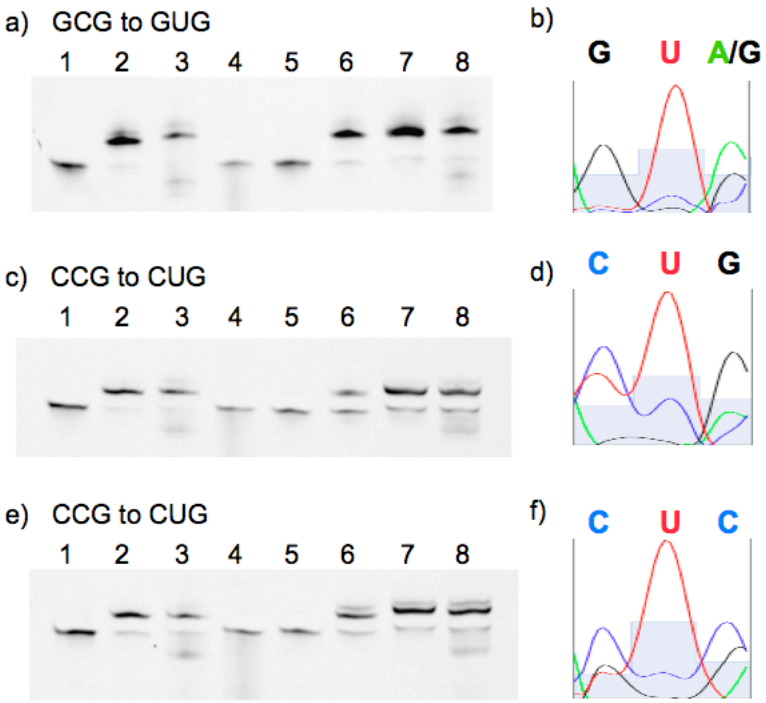

Fig. S1 Photochemical RNA editing of model RNAs, RNA(GCG), RNA(CCG) and RNA(CCC). (a) Denaturing PAGE analysis of the products after the each reaction step of photochemical RNA editing of RNA(GCG). Lane 1; RNA(GCG) + CNVK-ODN-1, Lane 2; Lane 1 + UV (366 nm, 0ºC, 30 sec), Lane 3; Lane 2 + heat (90ºC, 3.5 h), Lane 4; Lane 3 + UV (312 nm, 80ºC, 5 min), Lane 5; RNA(GUG) + CNVK-ODN-2, Lane 6; Lane 5 + UV (366 nm, 0ºC, 30 sec), Lane 7; Lane 2 + Lane 6, Lane 8; Lane 3+ Lane 6. (b) Result of Sanger sequence analysis of the product after the RNA editing of RNA(GCG). (c) Denaturing PAGE analysis of the products after the each reaction step of photochemical RNA editing of RNA(CCG). Lane 1; RNA(CCG) + CNVK-ODN-1, Lane 2; Lane 1 + UV (366 nm, 0ºC, 30 sec), Lane 3; Lane 2 + heat (90ºC, 3.5 h), Lane 4; Lane 3 + UV (312 nm, 80ºC, 5 min), Lane 5; RNA(CUG) + CNVK-ODN-2, Lane 6; Lane 5 + UV (366 nm, 0ºC, 30 sec), Lane 7; Lane 2 + Lane 6, Lane 8; Lane 3 + Lane 6. (d) Result of Sanger sequence analysis of the product after the RNA editing of RNA(CCG). (e) Denaturing PAGE analysis of the products after the each reaction step of photochemical RNA editing of RNA(CCC). Lane 1; RNA(CCC) + CNVK-ODN-2, Lane 2; Lane 1 + UV (366 nm, 0ºC, 30 sec), Lane 3; Lane 2 + heat (90ºC, 3.5 h), Lane 4; Lane 3 + UV (312 nm, 80ºC, 5 min), Lane 5; RNA(CUC) + CNVK-ODN-2, Lane 6; Lane 5 + UV (366 nm, 0ºC, 30 sec), Lane 7; Lane 2 + Lane 6, Lane 8; Lane 3 + Lane 6. (b) Result of Sanger sequence analysis of the product after the RNA editing of RNA(CCC).

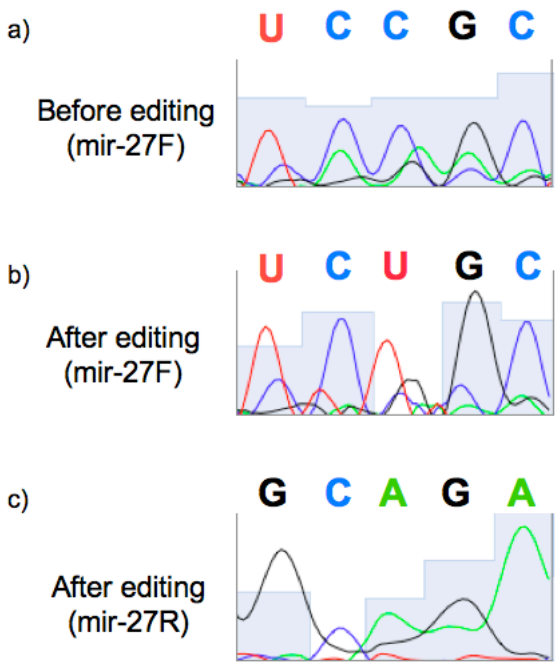

Fig. S2 Sequence analysis of the product after the photochemical RNA editing of miR-27b. (a) Before the photochemical RNA editing of mir-27b. Sequence was analyzed with forward sequence primer (mir-27F). (b) After the photochemical RNA editing of mir-27b. Sequence was analyzed with forward sequence primer (mir-27F). (c) After the photochemical RNA editing of mir-27b. Sequence was analyzed with reverse sequence primer (mir-27R).

References

1. Y. Yoshimura, K. Fujimoto, *Org. Lett.*, 2008, **10**, 3227.