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A photoactivatable microRNA probe for identification of microRNA targets and light-controlled suppression of microRNA target expression†

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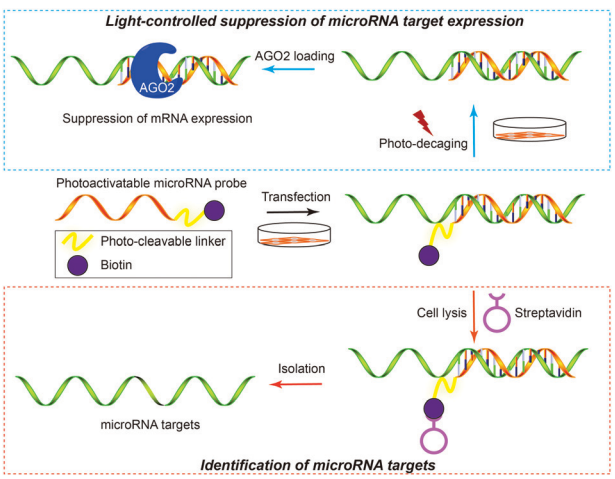
Here, we report a novel dual-functional microRNA (miRNA) probe, PA-miRNA, for miRNA target identification and light control of miRNA target expression. PA-miRNA is a miRNA mimic with a 3′-biotin tag linked *via* a photo-cleavable linker. Using PA-miR-34a, intracellular targets of miR-34a in HeLa cells were isolated and confirmed. Moreover, PA-miR-34a upon transfection into HeLa cells was inactive until light irradiation to break the photo-cleavable linker to release functional miR-34a. We demonstrated that miR-34a target expression as well as miR-34a-promoted cell apoptosis were regulated by PA-miR-34a in a photo-controllable manner.

The past two decades have witnessed growing research interest in microRNAs (miRNAs), which are a novel class of endogenous gene silencers.[1] The typical structures of miRNAs are single-stranded RNAs with ~22 nucleotides.[2] After binding to the 3′-untranslated regions (3′-UTRs) of target mRNAs and forming an RNA-induced silencing complex (RISC) with Argonaute 2 (AGO2)-included functional proteins, miRNAs guide suppression of mRNA translation.[3] Bioinformatic studies indicate that over 60% of human protein-coding genes are miRNA targets.[4] Therefore, miRNAs are extensively involved in both physiological and pathological processes.[1b,5] However, due to the complexity of miRNA-target mRNA networks and lack of suitable tools, miRNA-involved cellular processes are still far from understood. Practical strategies for identifying miRNA targets and connecting them with miRNA-regulated cellular behaviours are thus highly demanded.

Among reported experimental strategies for high-throughput identification of miRNA targets,[6] the pull-down method is straightforward and efficient. It employs biotinylated miRNA mimics as probes to isolate miRNA-associated targets through affinity purification.[7] However, although 3′-biotinlyated miRNA probes can be easily prepared, we and others have found that 3′-biotinylation hampers loading of these probes onto AGO2 and thereby causes loss of their function to suppress target gene expression.[8] Therefore, unified miRNA probes that retain both an affinity tag and biological activity for investigating miRNA targets as well as miRNA function still remain to be developed. Here, we report a dual-functional miRNA probe that allows not only identification of miRNA targets but also suppression of miRNA target expression upon external stimulus.

As shown in Scheme 1, we designed a photoactivatable miRNA probe (PA-miRNA) that is 3′-biotinylated miRNA with a photo-cleavable linker. Upon transfection into cells, PA-miRNA hybridizes with intracellular targets of miRNAs. The biotin tag in PA-miRNA then allows isolation of associated miRNA targets from cell lysis through streptavidin affinity purification, followed by identifying miRNA targets through high-throughput analysis.[6a] Alternatively, upon light stimulus on transfected cells, the photo-cleavable linker in PA-miRNA undergoes a photolysis reaction to release functional miRNA, causing turn-on of miRNA function to



Scheme 1 Schematic illustration of using a photoactivatable miRNA probe for identification of miRNA targets and photo-modulation of miRNA target expression.

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suppress target gene expression. Moreover, using light to activate PA-miRNA also permits spatial and temporal regulation of miRNA function, which is conducive to investigating miRNA-involved cellular processes.[9] Therefore, with this dual-functional miRNA probe, we are able to identify miRNA targets and explore miRNA function as well.

To demonstrate this hypothesis, we synthesized photoactivatable miR-34a (PA-miR-34a, Fig. 1a). miR-34a was selected due to its well-known tumor-suppressive function.[5,10] A photo-cleavable linker containing an *o*-nitrobenzyl moiety[11] was synthesized and used to conjugate biotin with the 3′-end of miR-34a. PA-miR-34a was easily prepared through solid-phase synthesis, followed by purification with high performance liquid chromatography (HPLC) and characterization by mass spectrometry (MS) (ESI†). Moreover, we also prepared unmodified miR-34a, scramble oligonucleotides (nc), 3′-biotinylated miR-34a without the photo-cleavable linker (Bio-miR-34a), and 3′-biotinylated nc with the photo-cleavable linker (PA-nc) for further uses as positive or negative controls.

We first studied the photolysis reaction of PA-miR-34a in PBS buffer (Fig. 1a). After irradiation with an 8 W hand-held 365 nm UV lamp for 0 to 2 min, the reaction mixtures were analyzed by HPLC and gel electrophoresis. As shown in Fig. 1b, the HPLC analysis result showed that the photolysis reaction occurred immediately after irradiation and completed after two minutes' irradiation. Gel-shift analysis confirmed this result (Fig S1, ESI†). We also collected the liberated miR-34a and validated its structure by MS (ESI†). It is worth noting that PA-miR-34a was stable in PBS for as long as 48 h when kept in the dark or under ambient light (Fig. S2, ESI†). The fast photolysis reaction suggests that photomodulation of the miR-34a function inside living cells is feasible by using PA-miR-34a and light irradiation.

We next moved to cellular studies of PA-miR-34a. PA-miR-34a was first used as the probe to isolate intracellular targets of miR-34a. PA-nc and nc were used as negative controls, whereas Bio-miR-34a was used as the positive control. We transfected HeLa cells with these probes, respectively. After 12 h, cell lysates were extracted and biotinylated species were isolated with streptavidin beads, followed by analysis of miR-34a and its targets with quantitative real-time PCR (qPCR). As shown in Fig. 2a, compared to those treated with nc or PA-nc, significant enrichment of miR-34a (~40-fold) was detected in pull-down samples from HeLa cells transfected with Bio-miR-34a or PA-miR-34a. Known miR-34a targets, including silent information

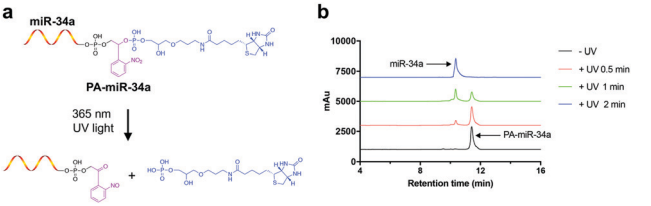

Fig. 1 Photolysis of PA-miR-34a. (a) Chemical structure of PA-miR-34a and its photolysis reaction upon irradiation with 365 nm UV light. (b) HPLC analysis of PA-miR-34a before and after UV irradiation for 0–2 min. An 8 W hand-held 365 nm lamp was used as the light source and the light intensity was 10 mW cm^{-2}.

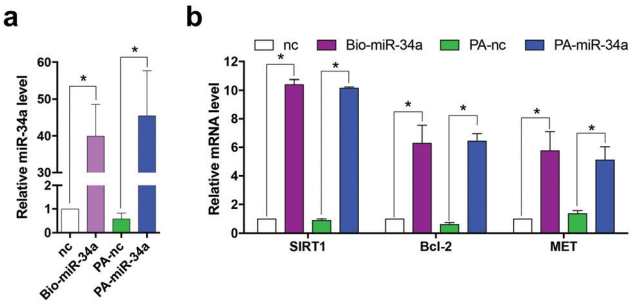

Fig. 2 Identification of miR-34a targets with PA-miR-34a. qPCR analysis of (a) miR-34a and (b) known miR-34a targets in pull-down samples from HeLa cells that were transfected with nc (100 nM), Bio-miR-34a (100 nM), PA-nc (100 nM), or PA-miR-34a (100 nM). GAPDH RNA levels served as the internal control. Data are shown as mean ± SEM (n = 3). *$P < 0.05$.

regulator 1 (SIRT1),[10] B-cell lymphoma-2 (Bcl-2)[12] and MET,[13] showed ~10-fold, ~6-fold and ~5-fold enrichments, respectively, in a pull-down sample of cells transfected with PA-miR-34a (Fig. 2b). Of note, the pull-down efficiency of PA-miR-34a was comparable to that of Bio-miR-34a (Fig. 2b). Collectively, these results demonstrated that PA-miRNA is an efficient probe for identification of miRNA targets.

Since 3′-biotin induces loss of miRNA function[8] and miR-34a could be liberated from PA-miR-34a by light irradiation (Fig. 1), we next investigated whether the intracellular function of PA-miR-34a in regulating miR-34a target expression could be induced by light irradiation. In addition, the chemical residue left on liberated miR-34a did not affect the intracellular function of miR-34a (Fig. S3, ESI†), warranting further regulation of miR-34a function through irradiation on PA-miR-34a. For readout of miR-34a function in living cells, we developed luciferase and green fluorescence protein (GFP) reporter systems by using the same method as previously reported (Fig. 3a).[8a,14] Briefly, 3′-UTRs of luciferase or GFP were respectively engineered to contain the 3′-UTR of SIRT1. Upon transfection of luciferase or GFP reporter genes into HeLa cells, functional miR-34a can bind to the 3′-UTR of the reporter genes and inhibit their expression. Therefore, we can directly read miR-34a function through measuring luciferase or GFP signals. We then co-transfected HeLa cells with luciferase reporter gene and PA-miR-34a. We also used nc, PA-nc, Bio-miR-34a or miR-34a instead of PA-miR-34a as negative or positive controls. After 48 h, luciferase signals were measured. In comparison with miR-34a, Bio-miR-34a and PA-miR-34a lost their function to repress the expression of miR-34a targets (Fig. 3b). These results were consistent with previous reports.[8]

To explore the effect of light irradiation on miR-34a target expression, we irradiated HeLa cells at 4 h post transfection with luciferase reporter gene and PA-miR-34a. The irradiation was performed with an 8 W hand-held 365 nm UV lamp for 0 to 5 min and luciferase signals were measured at 48 h post transfection. As shown in Fig. S4 (ESI†), PA-miR-34a function in regulating luciferase reporter expression was turned on immediately after irradiation and two minutes' irradiation was enough to fully recover its function. In addition, light irradiation did not significantly affect cell viability (Fig. S5, ESI†). Similar results

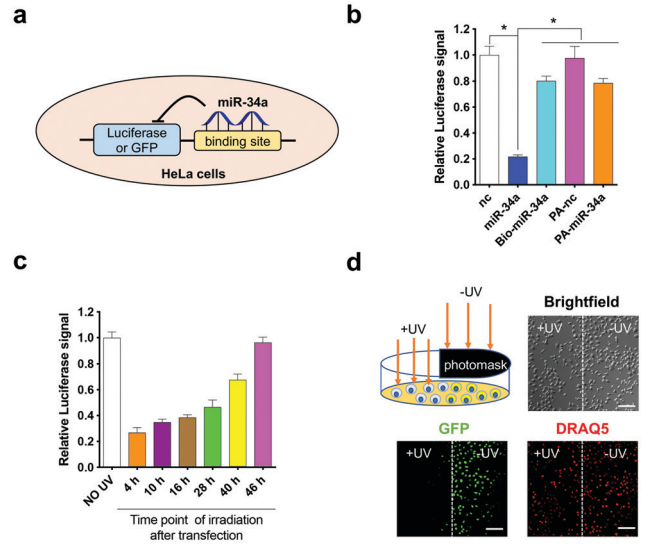

Fig. 3 Spatiotemporal regulation of miR-34a target expression by using PA-miR-34a and light irradiation. (a) Schematic illustration of luciferase and GFP reporter systems. (b and c) Relative luciferase signals from HeLa cells that were co-transfected with luciferase reporter gene (1 μg mL^{-1}) and PA-miR-34a (30 nM). (b) nc (30 nM), miR-34a (30 nM), Bio-miR-34a (30 nM) or PA-nc (30 nM) were used instead of PA-miR-34a as negative or positive controls. (c) At the time point of 4, 10, 16, 28, 40, and 46 h post transfection, HeLa cells were irradiated for 2 min. (d) Confocal fluorescence images of HeLa cells that were co-transfected with GFP gene (1 μg mL^{-1}) and PA-miR-34a (30 nM). At 4 h post transfection, HeLa cells were irradiated for 2 min by using a photomask. DRAQ5 was used to stain the nucleus. Scale bar: 100 μm. An 8 W hand-held 365 nm lamp was used as the light source and the light intensity was 10 mW cm^{-2}. Data are shown as mean ± SEM (n = 3). *P < 0.05.

were observed when using GFP reporter gene to read PA-miR-34a function under similar treatments and Bio-miR-34a function did not respond to light irradiation (Fig. S6, ESI†), confirming that recovery of PA-miR-34a function is induced by photolysis of the photo-cleavable linker in PA-miR-34a. These data suggested that miR-34a target expression can be regulated by PA-miR-34a and light stimulus.

Since light irradiation permits spatiotemporal regulation of biomoleucles,[9a] we further investigated whether miR-34a target expression could be regulated in a spatiotemporal manner through using PA-miR-34a and light irradiation. We co-transfected HeLa cells with luciferase reporter gene and PA-miR-34a. Two minutes' irradiation on HeLa cells was performed at 4, 10, 16, 28, 40 or 46 h after transfection, and luciferase signals were measured at 48 h post transfection. As shown in Fig. 3c, we observed a time-dependent manipulation of luciferase reporter gene expression. The gradual increase of luciferase signals could be attributed to the insufficient time for liberated miR-34a to inhibit luciferase expression. To explore the ability of PA-miR-34a to control miR-34a target expression in a spatial resolution, we co-transfected HeLa cells with GFP reporter gene and PA-miR-34a. At 4 h post transfection, HeLa cells were irradiated for 2 min, and a photomask was used to shield a part of the cells from irradiation. At 48 h post transfection, a fluorescence image of the HeLa cells was taken under a confocal microscope. Fig. 3d shows the fluorescence image, within which the irradiated area is left of the dotted line while the non-irradiated area is to the right side. GFP expression was repressed only within the irradiated area but not in the non-irradiated area, demonstrating successful spatial regulation of miR-34a target expression by using PA-miR-34a and light control.

It has been well established that miR-34a promotes cell apoptosis *via* targeting SIRT1.[10] Since miR-34a target expression can be regulated by PA-miR-34a in a light-controlled manner (Fig. 3), we further explored the potential of PA-miR-34a to induce light-dependent cell apoptosis. HeLa cells were transfected with PA-miR-34a and subject to two minutes' irradiation at 4 h post transfection. As negative or positive controls, we used nc, miR-34a, Bio-miR-34a or PA-nc instead of PA-miR-34a. At 48 h post transfection, we measured the protein levels of SIRT1 in HeLa cells as well as the apoptotic rates of HeLa cells. As shown in Fig. 4a, PA-miR-34a and Bio-miR-34a lost their function to suppress SIRT1 expression, whereas PA-miR-34a upon light irradiation restored its function to inhibit SIRT1. These results coincided well with those shown in Fig. 2b and again indicated that loss of the intracellular function of PA-miR-34a could be reversed through light irradiation. Flow cytometry analysis of cell apoptosis (Fig. 4b and c) further revealed that PA-miR-34a upon light irradiation led to a significant increase in cell apoptosis (~71%), which was comparable to that induced by miR-34a (~80%). Moreover, we also observed a dose-dependent regulation of cell viability by using PA-miR-34a and light irradiation (Fig. S7, ESI†). Taken together, these results demonstrated that light-inducible cell apoptosis is achievable through using PA-miR-34a and light irradiation.

In summary, we have developed a photoactivatable miRNA probe, PA-miR-34a, for identification of miR-34a targets and manipulation of miR-34a target expression. PA-miR-34a was prepared by linking biotin to the 3′-end of miR-34a through a photo-labile linker. Biotin served as both the affinity tag for pull-down of miR-34a targets and the caging moiety to block

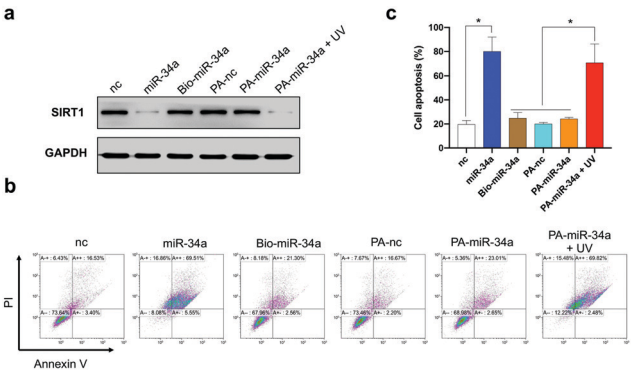

Fig. 4 Light-induced cell apoptosis by PA-miR-34a and light irradiation. (a) Western blotting analysis of the SIRT1 level in HeLa cells and (b and c) flow cytometry analysis of apoptosis of HeLa cells. (b) Representative results. (c) Quantitative analysis results. HeLa cells were transfected with nc (100 nM), miR-34a (100 nM), Bio-miR-34a (100 nM), PA-nc (100 nM), or PA-miR-34a (100 nM). HeLa cells transfected with PA-miR-34a were also irradiated with an 8 W hand-held 365 nm UV lamp (10 mW cm^{-2}) for 2 min at 4 h post transfection. Data are shown as mean ± SEM (n = 3). *P < 0.05.

miR-34a function, which could be restored upon light irradiation to remove biotin. Photolysis reaction of PA-miR-34a to liberate miR-34a completed within two minutes' irradiation, which is rapid enough for control of miR-34a function in living cells. By introducing PA-miR-34a into HeLa cells, we demonstrated successful isolation and confirmation of miR-34a targets. Moreover, we achieved spatiotemporal regulation of miR-34a target expression and light-inducible cell apoptosis. Overall, this study provides a practical tool for identification of miRNA targets and light-control of miRNA target expression. Using PA-miRNA to identify unknown miRNA targets and investigate the corresponding miRNA function is now underway in our group.

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Conflicts of interest

There are no conflicts to declare.

References

1 (*a*) D. P. Bartel, *Cell*, 2018, **173**, 20–51; (*b*) S. Lin and R. I. Gregory, *Nat. Rev. Cancer*, 2015, **15**, 321–333.
2 S. A. Gorski, J. Vogel and J. A. Doudna, *Nat. Rev. Mol. Cell Biol.*, 2017, **18**, 215–228.
3 (*a*) J. Liu, M. A. Carmell, F. V. Rivas, C. G. Marsden, J. M. Thomson, J. J. Song, S. M. Hammond, L. Joshua-Tor and G. J. Hannon, *Science*, 2004, **305**, 1437–1441; (*b*) G. Hutvagner and M. J. Simard, *Nat. Rev. Mol. Cell Biol.*, 2008, **9**, 22–32.
4 R. C. Friedman, K. K. Farh, C. B. Burge and D. P. Bartel, *Genome Res.*, 2009, **19**, 92–105.
5 R. Rupaimoole and F. J. Slack, *Nat. Rev. Drug Discovery*, 2017, **16**, 203–222.
6 (*a*) J. Li and Y. Zhang, *ExRNA*, 2019, **1**, 6; (*b*) J. Hausser and M. Zavolan, *Nat. Rev. Genet.*, 2014, **15**, 599–612.
7 (*a*) H. Baigude, Ahsanullah, Z. Li, Y. Zhou and T. M. Rana, *Angew. Chem., Int. Ed.*, 2012, **51**, 5880–5883; (*b*) A. Lal, M. P. Thomas, G. Altschuler, F. Navarro, E. O'Day, X. L. Li, C. Concepcion, Y. C. Han, J. Thiery, D. K. Rajani, A. Deutsch, O. Hofmann, A. Ventura, W. Hide and J. Lieberman, *PLoS Genet.*, 2011, **7**, e1002363; (*c*) M. Tan Shen, J. Lieberman, M. Tan Shen and J. Lieberman, *Methods Mol. Biol.*, 2016, **1358**, 211–228.
8 (*a*) J. Li, L. Huang, X. Xiao, Y. Chen, X. Wang, Z. Zhou, C. Zhang and Y. Zhang, *J. Am. Chem. Soc.*, 2016, **138**, 15943–15949; (*b*) J. Imig, A. Brunschweiger, A. Brummer, B. Guennewig, N. Mittal, S. Kishore, P. Tsikrika, A. P. Gerber, M. Zavolan and J. Hall, *Nat. Chem. Biol.*, 2015, **11**, 107–114; (*c*) Y. E. Guo and J. A. Steitz, *RNA*, 2014, **20**, 985–988.
9 (*a*) N. Ankenbruck, T. Courtney, Y. Naro and A. Deiters, *Angew. Chem., Int. Ed.*, 2018, **57**, 2768–2798; (*b*) T. Lucas, F. Schafer, P. Muller, S. A. Eming, A. Heckel and S. Dimmeler, *Nat. Commun.*, 2017, **8**, 15162; (*c*) F. Schafer, J. Wagner, A. Knau, S. Dimmeler and A. Heckel, *Angew. Chem., Int. Ed.*, 2013, **52**, 13558–13561.
10 (*a*) X. Xiao, X. Wang, Y. Wang, T. Yu, L. Huang, L. Chen, J. Li, C. Zhang and Y. Zhang, *Chem. – Eur. J.*, 2018, **24**, 2277–2285; (*b*) M. Yamakuchi, M. Ferlito and C. J. Lowenstein, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 13421–13426.
11 (*a*) Y. Ji, J. Yang, L. Wu, L. Yu and X. Tang, *Angew. Chem., Int. Ed.*, 2016, **55**, 2152–2156; (*b*) J. M. Govan, R. Uprety, M. Thomas, H. Lusic, M. O. Lively and A. Deiters, *ACS Chem. Biol.*, 2013, **8**, 2272–2282.
12 E. Slabakova, Z. Culig, J. Remsik and K. Soucek, *Cell Death Dis.*, 2017, **8**, e3100.
13 G. Misso, M. T. Di Martino, G. De Rosa, A. A. Farooqi, A. Lombardi, V. Campani, M. R. Zarone, A. Gulla, P. Tagliaferri, P. Tassone and M. Caraglia, *Mol. Ther.–Nucleic Acids*, 2014, **3**, e194.
14 (*a*) Z. Zhou, Q. Yi, T. Xia, W. Yin, A. A. Kadi, J. Li and Y. Zhang, *Org. Biomol. Chem.*, 2017, **15**, 2191–2198; (*b*) L. Huang, Y. Chen, L. Chen, X. Xiao, X. Wang, J. Li and Y. Zhang, *Chem. Commun.*, 2017, **53**, 6452–6455; (*c*) X. Xiao, J. Hu, X. Wang, L. Huang, Y. Chen, W. Wang, J. Li and Y. Zhang, *Chem. Commun.*, 2016, **52**, 12517–12520; (*d*) S. Tan, J. Li, X. Chen, W. Zhang, D. Zhang, C. Zhang, D. Li and Y. Zhang, *Cell Chem. Biol.*, 2014, **21**, 1265–1270; (*e*) J. Li, R. Kooger, M. He, X. Xiao, L. Zheng and Y. Zhang, *Chem. Commun.*, 2014, **50**, 3722–3724; (*f*) X. Wang, X. Xiao, B. Zhang, J. Li and Y. Zhang, *Chem. Commun.*, 2019, **55**, 2106–2109.

