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Fluorescence activation imaging of localization, distribution, and level of miRNA in various organelles inside cells

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ABSTRACT

This work reports an approach for imaging the localization, distribution, and level of miRNA in different organelles based on an activated fluorescence signal triggered by an alteration of the specific binding-induced conformation of the designed activatable probe. We selected miR-150 as an miRNA example to image its localization, distribution, and level in human cervical cancer cells (HeLa cells). The results indicate that miR-150 is localized and distributed in different subcellular organelles (mainly in mitochondria and lysosomes) and that its levels (actually its concentrations) in lysosomes are higher than those in mitochondria in both HeLa and MCF-7 cells. Moreover, the level of miRNA in cells is displayed in a height-dependent (in z-direction) manner. This approach can also be used to image the localization and distribution of various miRNAs (such as miR-150 and miR-214) in different organelles in cancer cells simultaneously. The probes exhibit high resistance to cellular endo- and exonucleases, with high specificity; the capability of avoiding false signals, with a high signal-to-background ratio; and a good ability to operate in complicated environments. The developed approach may provide a useful tool for studying the localization and distribution and evaluating the level of multiple tumor-related miRNAs in cells.

1. Introduction

MiRNAs (miRNAs) have been shown to function as prognostic and diagnostic biomarkers, as well as therapeutic targets, for a wide range of human diseases, such as cancers [1–4]. Cancer-specific miRNA fingerprints have been identified in several types of cancers [5–7]. For example, miR-141 has been revealed to be overexpressed in prostate cancer cells (22 Rv1 cells) and connected to prostate cancer development [8]; the amount of let-7 family in lung cancer is significantly lower than in normal tissue [7]. Thus, miR-141 and let-7 could be used as biomarkers for the diagnosis of prostate and lung cancers, respectively. Moreover, miRNAs in certain organelles may regulate the structures and functions of these organelles [9]. For example, miRNAs in mitochondria affect mitochondrial metabolism, participate in the regulation of mitochondria-related apoptosis, and control mitochondrial morphology [10,11]. Therefore, modulation of miRNA levels may provide a new diagnostic and therapeutic approach for mitochondria-related diseases. However, to efficiently use miRNA biomarkers for accurate early diagnosis and to modulate their levels in specific organelles, the monitoring of their localizations, distributions, and levels in different organelles inside cancer cells under in vivo conditions are required.

Currently, fluorescence in situ hybridization (FISH) is a common approach (using DNA-based fluorescence probes) to assay and image miRNAs in cells, tissues, and tumors [12–26]. However, the structures of these probes are complicated and their designs and preparations are not straightforward. To achieve the detection signal, several DNA strands are usually required, leading to a low efficiency of use of the probes and limiting their uses in clinical diagnosis [27]. Moreover, there are no reports containing imaging of the localizations, distributions, and levels of miRNAs in different organelles.

This work addresses this issue and reports an approach for the fluorescent imaging of the localizations, distributions, and levels of miRNAs in various organelles. The imaging is based on the activated fluorescence signal of a designed activatable probe. We selected miR-150 and miR-214 as miRNA examples to image their localizations, distributions, and levels in human cervical cancer cells (HeLa cells) and human breast cancer cells (MCF-7). The results indicate that miRNAs (miR-150 and miR-214) are localized and distributed in different subcellular organelles (mainly in mitochondria and lysosomes), and their levels in lysosomes are higher than in mitochondria, in both HeLa and MCF-7 cells. Moreover, the levels of miRNAs in cells display a height-

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dependent (in the z-direction) manner.

This approach offers several advantages. The probe has a simple structure, which can be easily designed and prepared, and has considerable resistance to cellular endo- and exonucleases and a high signal-to-noise ratio. The recorded signal is caused by the specific target-miRNA-binding-triggered conformational change in the probe, with high specificity the capability of avoiding false signals arising from the nonspecific adsorption of interferents.

2. Materials and methods

2.1. Fluorescence assays

In a typical assay, cells (~1 × 10^5 cells mL^{-1}. Refer to Supporting information for chemicals and the detailed procedures for cell culture) were cultured in a medium containing activatable-P150 probe (0.25 μM. The detailed sequence of the probes and targets are listed in Table S1) for 24 h at 37 °C in a CO_2 atmosphere. After that, the adherent growth cells were digested with trypsin (0.25%) for 30 s, and the suspended cells were transferred into a centrifuge tube, washed with PBS (pH 7.4) three times and re-suspended in PBS for fluorescence measurements. Fluorescence spectra were collected using a FluoroSENS fluorescence spectrophotometer (Gilden Photonics). The emission spectra of Cy5 were recorded under an excitation of 638 nm.

2.2. Confocal laser microscopic imaging

The cells were first transferred into a 35-mm Petri dish with 10-mm bottom wells in culture medium and were incubated with the probe (0.25 μM) at 37 °C for 24 h. Each well was washed three times with PBS before imaging. The images were recorded with a confocal laser scanning microscope (CLSM, MRC-1024, Bio-Rad, Ltd.) equipped with an oil immersion 100 × objective. The CLSM images of Cy5 and Cy3 were collected under excitations of 638 and 543 nm, respectively. The intensity was analyzed using the NIS-Elements AR software provided with the instrument.

3. Results and discussion

The aim of this work was to develop an approach for the fluorescent imaging of the localizations, distributions, and levels of miRNAs in various organelles based on the activated fluorescence signal of an activatable probe. The signal is triggered by an alteration in the specific-binding-induced conformation of the probe (Fig. 1), which consists of two fragments: a target-miRNA-recognized sequence for specifically recognizing the targets and an extending spacer for folding of the probe into a hairpin structure, facilitating fluorescent resonance energy transfer (FRET) between the labeled fluorophore (at the 5′-terminus) and quencher (at the 3′-terminus). Before interaction with miRNAs (for example, miR-150) in cells, the fluorescence of the fluorophore (Cy5, labeled at 5′-terminus of the probe. Table S1) is quenched due to the close proximity of the quencher (BHQ2, labeled at the 3′-terminus) to the fluorophore. After interacting with miR-150, however, the fluorescence signal of Cy5 is significantly activated. Therefore, the localizations, distributions, and levels of miRNAs in various organelles can be imaged by recording the activated fluorescence signals. Furthermore, by designing different probes and selecting various fluorophore/quencher combinations, we can achieve simultaneous imaging of multiple miRNAs in cells.

We first performed a number of in vitro experiments to confirm the binding features of the probe with target miRNA. Binding studies were first performed with a perfectly matched target (miR-150), and then with the single-base mismatched target (one-base mismatch miR-150) and other miRNAs (miR-21 and miR-34b). The results in Fig. S1 show that the probe is specifically bound to miR-150 and generated a strong fluorescence signal (activated fluorescence). The simulation results

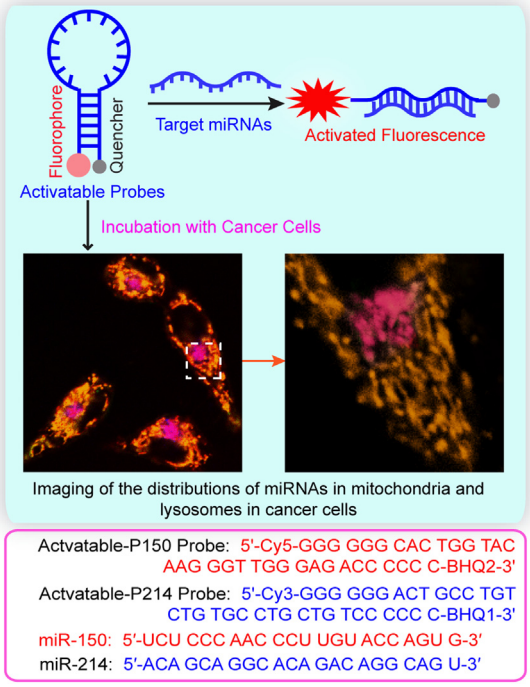

Fig. 1. Schematic illustration of imaging of localizations, distributions, and levels of miRNAs in different organelles in cells using the activatable DNA-based fluorescence probe.

(simulated by the NUPACK program) also indicate that the formation of the duplex between the probe and miR-150 is thermodynamically more favorable than the stem-loop structure of the probe because the free energy of the duplex -31.21 kcal mol^{-1} is much more negative than that of the probe (–4.98 kcal mol^{-1}) (Fig. S2). In contrast, the fluorescence signals do not obviously change in the presence of other miRNAs (single-base mismatched target, miR-21, and miR-34b), implying that the probe cannot be effectively hybridized. The native polyacrylamide gel electrophoresis (PAGE) analysis also verifies the specific hybridization of the probe with miR-150 but not with other interference sequences (Fig. S3), validating the results obtained from fluorescence spectra (Fig. S1). These results demonstrate that the probe retains the high sequence specificity offered by the conformational constraint of the stem-loop structures.

The binding features of the probe with a target may be affected by the pH of the medium. Moreover, the pH inside cells varies across organelles; for example the characteristic pH of the mitochondria and lysosomes is ~8.0 and 4.7–6.5 [28], respectively. To confirm the effects of pH on the binding characteristics of the probe, binding studies were also performed in acidic medium (pH 6.0, the characteristic pH of lysosomes) and basic medium (pH 8.0, the characteristic pH of the mitochondria). The results depicted in Fig. S4 suggest that the medium pH has a negligible effect on the binding of the probe with the target, since the activated fluorescence signals remain invariant at pH 6.0–8.0.

Furthermore, the presence of the mitochondria (Mito@tracker green) and lysosome tracker (Lyso@tracker blue) does not affect the binding of the probe with a target because the activated fluorescence signals of the probe are not altered after addition of these trackers into the system (Fig. S5).

The biocompatibility, nuclease stability, and cellular uptake of the probes were then studied. The MTT assays indicated that the cells could retain more than 95% viability after they were incubated with the probe for 24 h at concentrations up to 1 μM (Fig. S6), implying that the probe showed almost no cytotoxicity or side effects in live cells. The nuclease stability was examined by incubating the probe (0.25 μM) with DNase I-containing PBS (DNase I is a common endonuclease in cells)