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Biophysical Chemistry 122 (2006) 1 – 4

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

Magnetic force microscopy analysis of apoptosis of HL-60 cells induced by complex of antisense oligonucleotides and magnetic nanoparticles

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Received 26 October 2005; received in revised form 10 January 2006; accepted 10 January 2006
Available online 24 February 2006

Abstract

Magnetic force microscopy (MFM) has been employed to observe antisense oligonucleotides (ASOs)-coupled silica-coated magnetic iron oxide nanoparticles (SMNPs) internalized into human leukemia (HL-60) cells. The experiment demonstrated that the ASOs-coupled SMNPs delivery into the cells really occurred. The nanoparticles were internalized into the cells and the apoptotic topography can be directly visualized simultaneously with MFM technology. These present observations offer direct morphology evidence on studying the apoptosis of tumor cells and provide useful information for better design of new diagnostic and therapeutic tools in tumor treatment.

Keywords: Human leukemia cells; Magnetic force microscopy; Morphologic image; Magnetic image; Antisense oligonucleotides; Apoptosis

Antisense technique is an important area of research in cancer-associated treatment. Compared with conventional cancer chemotherapy, antisense therapy has less cytotoxic side-effects based on the identification of cancer-associated molecular sites, which could allow the selective targeting of cancer cells without suppressing normal cells [1–3]. The principle of this technology is the sequence-specific binding of an antisense oligonucleotide (ASOs) to target mRNA, thereby preventing gene translation [4,5]. ASOs are one of such class of agents for inhibiting gene expression and resulting in cell apoptosis.

The application of magnetic nanoparticle–ligand targeting systems for drug delivery is of particular interest. Acting as a vehicle to specifically deliver cancer-fighting drugs to tumors, magnetic nanoparticles show great promise for research towards clinical diagnostic and therapeutic applications [6–9]. ASOs–SMNPs conjugation is thus a considerably exciting targeting delivery system for specific cellular uptake and selective tumor targeting.

Magnetic force microscopy (MFM) is an offspring of atomic force microscope (AFM) and employs a fine magnetic tip attached to a flexible cantilever. It is particularly suitable for magnetic recording systems, magnetic thin-film media on textured substrates or the iron–magnetism studying [10]. Up to date, relatively little has been applied in observing the morphology and the structural details of cells by MFM. Utilizing TEM and AFM to characterize the biophysical properties of cells after the cell uptake has previously been reported [11,12]. However, simultaneous observation of the surface and the nanoparticles in the cell without destroying it proved to be non-trivial. In this report, we utilized MFM to observe the uptake of ASOs–SMNPs conjugation into HL-60 cells for the first time.

ASOs-coupled SMNPs were synthesized by the reaction of 19-mer-ASOs with thiol-modified SMNPs according to our previously reported method [13]. Aliquots of human leukemia cells (HL-60, 10^6/mL) were cultured in the presence of ASOs–SMNPs and ASOs-free SMNPs (both are 8 mg/mL) respectively under physiological relevant conditions. After a period of time, a phenomenon of the ASOs–SMNPs complex and SMNPs internalization into cells were visualized by a

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doi:10.1016/j.bpc.2006.01.003

multimode MFM with Nanoscopy IIIa control system (Digital Instruments). Fig. 1 shows MFM images of HL-60 cells treated with ASOs-coupled SMNPs after 48 and 72 h, respectively. In Fig. 1, volume 1 displays a morphologic image, which was submitted to MFM observations using the tapping mode by the first scanning. While the second scanning was performed using the lift mode that lifts the fine magnetic tip to a height of 20 nm, we obtained magnetic images (volumes 2 and 3). As shown in volumes 2 and 3, ASOs-coupled SMNPs are clearly visualized in the cell. When the tip scanned the cell from top to the bottom, the nanoparticles lingered in the top of the cell (volume 2); however, if a reverse direction scanning was performed, they are deposited in the bottom (volume 3). Therefore, it can give evidence that the ASOs-coupled SMNPs have successfully entered into the HL-60 cells, which is an important precondition for a specific interaction between ASOs and telomerase [6].

In Fig. 1, volume 1 shows distinct morphologic images of the cell uptake after 48 and 72 h. It can be seen from a1 that the brightest region moved to one side of the cell after 48 h incubation. This phenomenon indicates that the intracellular nuclear excursion occurred, which is one of the characteristics of apoptosis. In addition, there were some bubbles on other cells, as shown in b1, which displays that the cells were in apoptosis as well. c1 shows another dramatically different photograph when the cells were grown with ASOs-coupled SMNPs for 72 h. The cell displays a characteristic morphology changes comprising of cell transfiguration, membrane bubbling, many cavitations on the cell surface, etc., which indicate that the apoptotic cells were in advance stage. The MFM images captured show that the different extents of apoptotic cells were observed with varied incubation time under interaction with ASOs-coupled SMNPs.

To find out whether the mortality of the HL-60 cells was due to the triggering by ASOs or SMNPs, we carried out another two parallel control experiments with blank incubation and ASOs-free SMNPs incubation after 48 h as well as ASOs-coupled SMNPs incubation. As shown in Fig. 2, the HL-60 cells treated with 8 mg/mL ASOs–SMNPs (c1) showed a visible apoptotic morphology. In contrast, cells treated with ASOs-free SMNPs (b1) as well as untreated HL-60 cells (a1) did not show apoptotic morphology. Undoubtedly, the ASOs can induce the apoptosis of HL-60 cells.

In order to detect ASOs-induced apoptosis, a further analysis by flow cytometry was carried out. As shown in Fig. 3, the HL-60 cells treated with 8 mg/mL ASOs–SMNPs showed a hypodiploid DNA peak from the diploid DNA. This peak is the result of the reduced DNA content characteristic of apoptotic cells. The percentage of the cells was about 23.83%. While cells treated with ASOs-free SMNPs as well as untreated HL-60 cells

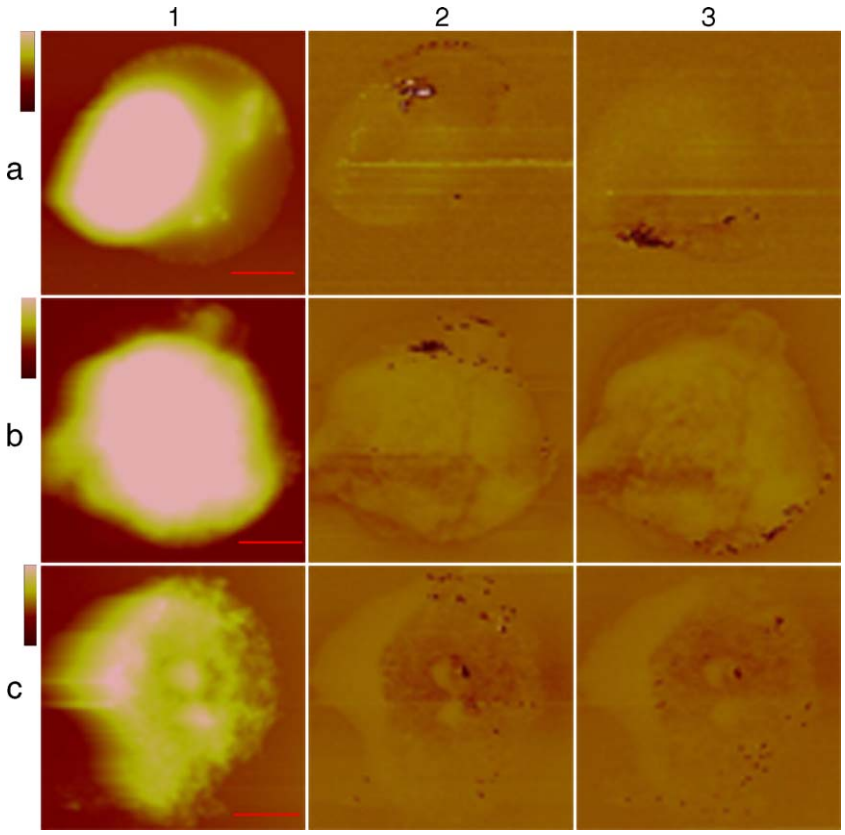

Fig. 1. MFM images of HL-60 cell treated with ASOs-coupled SMNPs after 48 h and 72 h, respectively. Volume 1 is morphologic images. Volumes 2 and 3 are magnetic images; volume 2 shows the tip scanned from the top to the bottom; volume 3 shows the tip scanning from the bottom to the top. Scale bars are (a) 3.5, (b) 2.4 and (c) 4.3 μm. Color bars are (a) 1.5, (b) 2.5 and (c) 1.5 μm. From the morphologic image, the cell displayed different extents of apoptotic characteristic morphology. From the magnetic image, the particles can be clearly seen in the cell.

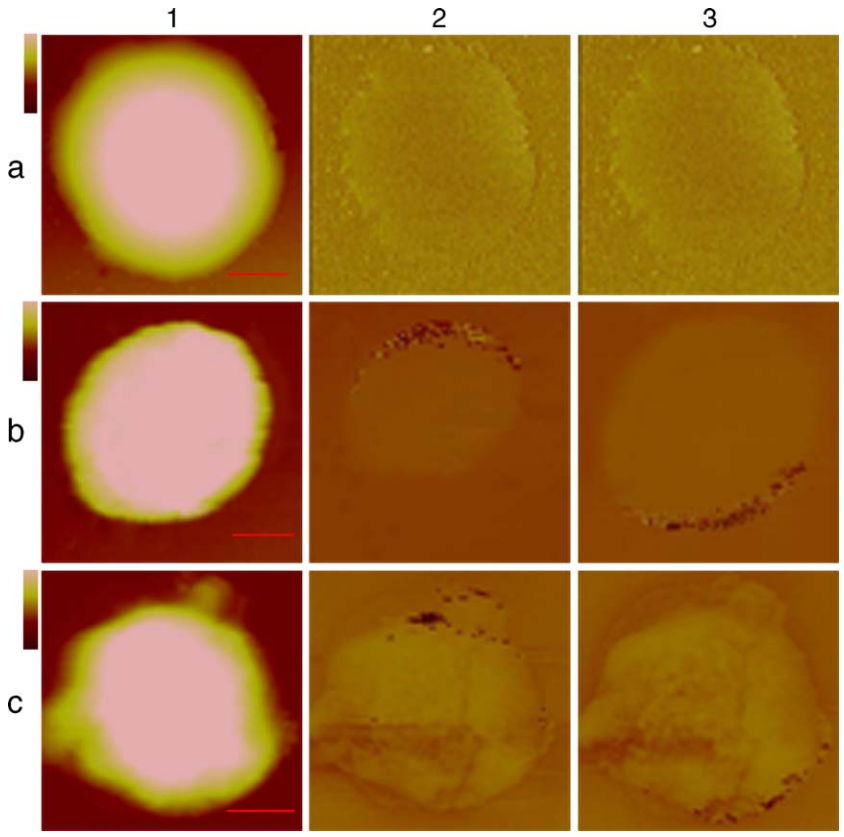

Fig. 2. MFM images of HL-60 cell untreated (row a) and treated with ASOs-free SMNPs (row b) and treated with ASOs-coupled SMNPs (row c) after 48 h. Volume 1 is a morphologic image; volumes 2 and 3 are magnetic images; volume 2 shows the tip scanned from the top to the bottom; volume 3 indicates the scanning from the bottom to the top. Scale bars are (a) 1.8, (b) 3.7 and (c) 2.4 μm. Color bars are (a) 1.5, (b) 4.0 and (c) 2.0 μm. ASOs-loaded SMNPs treated HL-60 cells displayed distinct apoptosis, while the ASOs-free SMNPs treated and untreated cells did not show apoptotic morphology.

did not show the visible hypodiploid DNA peak. The percentages of the cells were 11.71% and 5.02%, respectively. These data significantly verified that ASOs are a potent growth inhibitor for HL-60 cells.

The reported 19-mer antisense oligonucleotide in this paper was complementary to a 19 nucleotide-long sequence, which belongs to the template region of the telomerase RNA component hTR. Its combination with the telomerase RNA through Watson-Crick base pairing rule results in the depression of the telomerase activity, therefore inhibiting the gene expressing and controlling the cell proliferation and leading to cell apoptosis.

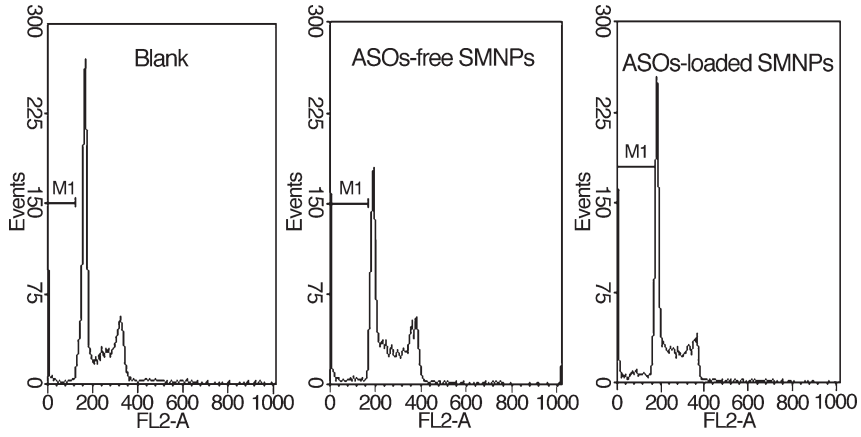

Fig. 3. Flow cytometry analysis. DNA fluorescence histograms of propidium iodide-stained HL-60 cells without treatment and treated with 8 mg/mL ASOs-free SMNPs or the same concentration of ASOs-loaded SMNPs. After culturing the trisection cells for 2 days, cells were harvested, washed in PBS, fixed in 75% pre-cold ethanol at 4 °C for at least 24 h, Rnase (20 μg/mL) treated in PBS at 37 °C in the dark for 30 min and stained with propidium iodide (20 μg/mL). The samples were read in a FACSCALIBUR cytometer (Becton-Dickinson) and the apoptotic rate of the HL-60 cells were calculated using a software for DNA analysis. Apoptotic cells are indicated by the M1 bar.

SMNPs-mediated administration system has long been considered to be a high-efficiency target delivery. Antisense oligonucleotides technology is one of the most promising therapeutic strategies to prevent the progress of diseases through inhibiting the specific gene expression. ASOs–SMNPs conjugation is just the excellent combination of them. Our present data demonstrated that the ASOs-coupled SMNPs delivery into the cells really occurred and, at the same time, the nanoparticle internalization into cells and the apoptotic topography of cells could be directly visualized using MFM technology. These present observations offer direct evidence on studying the apoptosis of tumor cells and provide useful information for better design of new diagnostic and therapeutic tools in tumor treatment.

Acknowledgment

We thank the National Natural Science Foundation of China for financial support of this research (No. 20443005) and the Nanotechnology Special Projects of Shanghai (No. 0352 nm 123).

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