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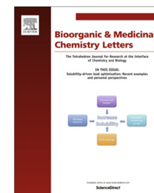

Photoinduced apoptosis using a peptide carrying a photosensitizer

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ABSTRACT

A novel molecule, TatBim-Alexa, consisting of the HIV1 Tat cell-penetrating peptide, the Bim apoptosis-inducing peptide, and Alexa Fluor 546 was synthesized for photoinducion of apoptosis. The Alexa Fluor 546 was used as a photosensitizer and covalently attached at the C-terminus of TatBim peptide by the thiol–maleimide reaction. Photo-dependent cytosolic internalization of TatBim-Alexa and photo-dependent apoptosis using TatBim-Alexa were demonstrated in several kinds of mammalian cells including human cancer cell lines.

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Methods to spatiotemporally control cellular functions by light are quite useful for analyzing biological systems. For example, optogenetic methods using rhodopsin proteins have revealed various functions of nervous systems.[1,2] Photochemical internalization (PCI) is the method used to photo-dependently deliver various macromolecules, such as peptides and nucleic acids, into cytosol.[3–5] This strategy has been used to photoinduce cellular functions, such as RNA interference,[6] and targeted cell death.[7]

In this study, a PCI-based method to photoinduce apoptosis was developed. As a photoinducer, we designed the TatBim-Alexa molecule, which is a conjugate of Tat cell-penetrating peptide (CPP) from the HIV-1 TAT protein,[8] the BH3 domain derived from Bim apoptosis-inducing protein,[9,10] and the Alexa Fluor 546 dye (Alexa546). Alexa546 can be used as a photosensitizer when it is attached to a Tat fusion protein/peptide.[11–13] We assumed that TatBim-Alexa would enter into cells by the endocytic pathway, would be entrapped in endosomes, and then would escape from the endosomes and induce apoptosis by photoirradiation. These assumptions are supported by precedents using other fusion molecules including Tat and Alexa546. For example, attachment of Alexa546 to an RNA carrier protein TatU1A, which is a fusion protein of Tat and U1A RNA binding protein, added photo-dependent endosomal escape ability to TatU1A.[11,13] The fusion peptide of Tat and Bim peptides (TAT-Bim) is known to induce apoptosis in cancer cells.[14] TAT-ODD-Caspase 3 fusion protein,[15] Bak-octaarginine fusion peptide,[16] and Tat-FOXO3 fusion protein[17] can also induce apoptosis. However, these peptides/proteins cannot spatially control apoptosis. Thus, we prepared TatBim-Alexa to enable spatiotemporally controlled apoptosis by the PCI strategy.

The TatBim-Alexa molecule was prepared as follows. TatBim-C peptide (H-RKKRRqRRREIWAQELRRIGDEFNAYYARgc-NH_2), in which amino acid residues in lower case are different from those in previously described TAT-Bim,[14] was prepared by a conventional Fmoc-based solid-phase peptide synthesis. TatBim-C contains a cysteine at the C-terminus for reaction with a maleimide group of the Alexa dye. The TatBim-C peptide was reacted with Alexa Fluor 546 C5 maleimide (Invitrogen, USA) to generate TatBim-Alexa, and TatBim-Alexa was purified by a reversed-phase HPLC.[18] The purified TatBim-Alexa was analyzed by SDS–PAGE (Fig. S1), which showed that the purified TatBim-Alexa fraction did not include the free Alexa dye. The labeling efficiency of TatBim-Alexa was calculated from the absorbance at 280 nm, which includes absorbances of TatBim peptide and Alexa546, and at 556 nm (absorbance of Alexa546). The Alexa546 attachment efficiency to the TatBim-C peptide was adjusted to 50% using the free TatBim-C peptide, and this was used in all of the following experiments. An inactive mutant of TatBim-Alexa [TatBim(inactive)-Alexa (Alexa546 attachment: 50%)] was also prepared as described above using a TatBim(inactive)-C peptide (H-RKKRRQRRREIWIAQEARRI-GAEFNAYYARGC-NH_2).

To examine cellular internalization of TatBim-Alexa, mammalian cells were treated with the molecule and visualized using a fluorescence microscope.[19] As shown in Figure 1, TatBim-Alexa was internalized by the Chinese hamster ovary (CHO) cells and human cervical cancer HeLa cells. Before irradiation, cytoplasmic dotted localization of TatBim-Alexa was observed in CHO and HeLa cells (Fig. 1A, B), suggesting that TatBim-Alexa molecules entered

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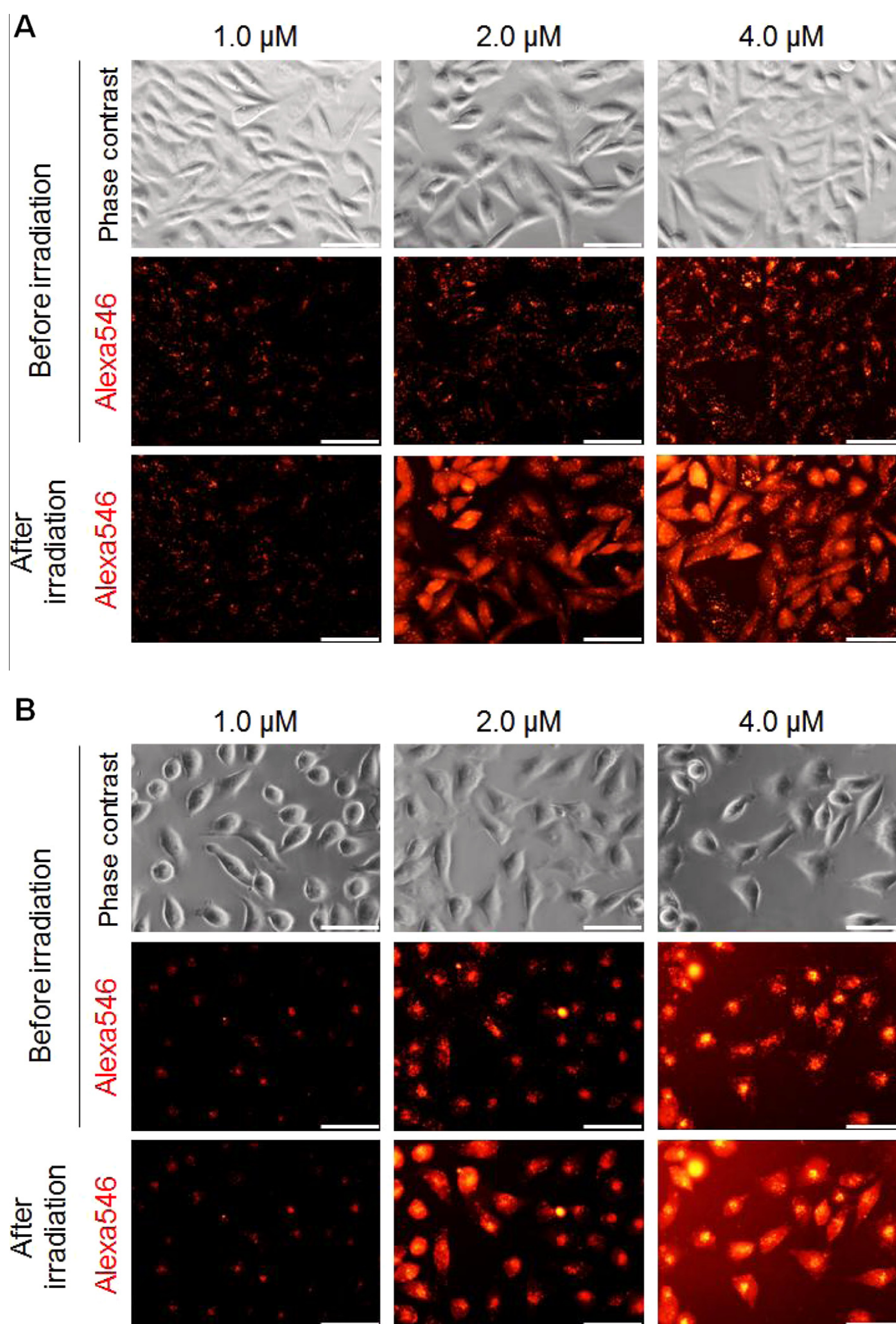

Figure 1. Internalization of TatBim-Alexa by mammalian cells. These cells were treated with TatBim-Alexa (1.0, 2.0 or 4.0 μM) and irradiated. Images of CHO (A) and HeLa cells (B) before and after irradiation are shown. Scale bars indicate 50 μm.

into cells by endocytosis and were entrapped in endosomes as previously described using Tat-fusion molecules.[11,20] Nuclear localization was also observed in HeLa cells (Fig. 1B). After irradiation of the cells with light at a peak wavelength of 530–550 nm and at a fluence of 20 J/cm^2, cytoplasmic dispersion of TatBim-Alexa was observed after irradiation when the cells were treated with more than 2 μM of TatBim-Alexa (Fig. 1A, B). No effect of photoirradiation was observed at the TatBim-Alexa concentration of 1.0 μM, which is likely to be due to insufficient photosensitizer concentration. Therefore, TatBim-Alexa (more than 2 μM) can photo-dependently enter into the cytoplasm of mammalian cells.

Next, we attempted to photoinduce apoptosis using TatBim-Alexa in mammalian cells including CHO, HeLa, and epithelioid carcinoma Panc 02.03 cells. To induce apoptosis, cells were treated with TatBim-Alexa for 2 h at 37 °C, irradiated, and further incubated for 5 h at 37 °C.[21] Apoptosis was detected using NucView™ 488 Caspase-3 Assay Kit (Biotium, USA), which is a marker of early apoptosis. The apoptotic cells were visualized using the Olympus IX51 fluorescence microscope with 40× objective lens (λ_{ex} = 470–490 nm). Cells incubated at 37 °C for 5 h with medium containing 1 μM staurosporine were used as a positive control. The treatment with TatBim-Alexa followed by irradiation successfully induced apoptosis in CHO, HeLa and Panc 02.03 cells (Fig. 2). In contrast, without irradiation, TatBim-Alexa did not induce apoptosis above a very low baseline level in these cells. Induction of apoptosis was also not observed in non-irradiated cells treated with TatBim or Alexa546, and in the cells subjected to irradiation only (without TatBim-Alexa treatment). The apoptosis efficiency of HeLa cells treated with TatBim-Alexa was estimated as shown in Figure 3. Previous results showed that TAT-Bim at high

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