

Original Article

Gallic acid-mitochondria targeting sequence-H₃R₉ induces mitochondria-targeted cytoprotection

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ABSTRACT The development of selective targeting of drug molecules towards the mitochondria is an important issue related to therapy efficacy. In this study, we report that gallic acid (GA)-mitochondria targeting sequence (MTS)-H₃R₉ exhibits a dual role as a mitochondria-targeting vehicle with antioxidant activity for disease therapy. In viability assays, GA-MTS-H₃R₉ showed a better rescue action compared to that of MTS-H₃R₉. GA-MTS-H₃R₉ dramatically exhibited cell penetration and intercellular uptake compared to MTS and fit escape from lysosome release to the cytosol. We demonstrated the useful targeting of GA-MTS-H₃R₉ towards mitochondria in AC16 cells. Also, we observed that the antioxidant properties of mitochondrial-accrued GA-MTS-H₃R₉ alleviated cell damage by reactive oxygen species production and disrupted mitochondrial membrane potential. GA-MTS-H₃R₉ showed a very increased cytoprotective effect against anticancer activity compared to that of MTS-H₃R₉. We showed that GA-MTS-H₃R₉ can act as a vehicle for mitochondria-targeting and as a reagent for therapeutic applications intended for cardiovascular disease treatment.

INTRODUCTION

Cardiovascular disease, including myocardial infarction, myocardial ischemia, and heart failure are a major cause of morbidity and mortality worldwide [1]. These diseases include risk factors such as ageing, hypertension, and obesity, which lead to heart failure presenting as a terminal clinical signal by left ventricular dysfunction due to progressive pathological remodeling characterized by endothelial dysfunction, cardiac hypertrophy, and interstitial fibrosis [2]. However, current treatments such as pharmacologic agents and invasive surgical therapy only delay the progression towards heart failure and the only cure for these diseases is cardiac transplantation [3,4]. Consequently, strategies with better outcomes are needed for these diseases. Nanotechnology has provided important information for therapeutics in various fields

including medicine and biology. Nanomaterials are typically in the range of 1–100 nm to specifically target delivery of peptides, protein, drug, and genes to desired therapy sites [5,6]. The commonly used nanomaterials are liposomes, nanoshells, polymeric micelles, dendrimers, graphene, and bacterial nanoparticles [7].

Efficient nanomaterials as drug carriers are stable, biodegradable has easy surface modification and can be used in organelle-specific delivery such as towards the mitochondria and nucleic acid for various diseases [8]. Mitochondria are very complex organelles, which function as powerhouses for cellular energy and are related to energy metabolism, redox regulation, apoptosis, and reactive oxygen species (ROS). Dysfunctional mitochondria caused by mitochondrial oxidative damage due to impaired mitochondrial ATP synthesis are related to many human disorders including neurodegenerative disease, cancers, diabetes, and isch-



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emia-reperfusion injury [9,10]. Until now, a few mitochondrial delivery vehicles have been used for high selective delivery into mitochondria such as triphenylphosphonium, dequalinium, protein transduction domain peptides, and mitochondria targeting sequence (MTS) peptides. However, an important issue regarding safe and efficient functionalization of vehicles to organelle-specific targeting properties remains [11,12]. Among the various mitochondrial targeting vehicles, the MTS which is composed of 15–50 amino acids with amphipathic helical structural feature that can deliver drugs into mitochondria by mitochondrial transport machinery has been studied. It has very low cellular uptake despite having benefits such as simple production and tunable modification for delivery of bioactive cargos to mitochondria [13,14]. According to a report, MTS-H₃R₉ could be a potential mitochondrial delivery vehicle with improved mitochondria selective targeting efficiency as well as a therapeutic agent, which assists cellular penetrating moieties across the plasma membrane by positively charged conjugation of histidine and arginine residues [15–17].

We characterized an MTS-based vehicle system, GA-MTS-H₃R₉ to demonstrate that such organ specific delivery vehicles can act as an effective drug. We used MTS-H₃R₉ with gallic acid (GA, 3,4,5-trihydroxy benzoic acid) as a model drug (GA-MTS-H₃R₉). GA, a polyphenyl natural product, is found in grapes, black teas, berries, and olive oil. It was known to have anticancer, anti-inflammatory, antioxidant, antibacterial activities and therefore has attracted attention in drug, food, cosmetic, and biomedical fields [18,19]. We aimed to develop a valid mitochondrial carrier and establish the mechanism of cellular function by conjugating GA with MTS peptide-H₃R₉, denoted as GA-MTS-H₃R₉ in AC16 cells, derived from human ventricular heart tissues. We demonstrated that MTS-H₃R₉ showed enhanced mitochondria-targeting efficiency and cellular uptake with or without GA compared to that of unmodified MTS. Here, we showed that GA-MTS-H₃R₉ exhibits low cytotoxicity, rescued ROS generation, and decreased apoptotic activity *via* mitochondrial related pathway compared with that of MTS-H₃R₉ in AC16 human cardiomyocyte cell line. We suggest that the dual effects of GA-MTS-H₃R₉ as a drug delivery carrier and a therapeutic agent warrant further studies eventually aiming at developing drugs for mitochondria-functional regulation to be used against cardiovascular disease.

METHODS

Synthesis of peptides

All peptides (K [gallic acid], MTS, MTS-H₃R₆, MTS-H₃R₉, GA-MTS-H₃R₆, GA-MTS-H₃R₉, FITC-K [gallic acid], FITC-MTS, FITC-MTS-H₃R₆, FITC-MTS-H₃R₉, FITC-GA-MTS-H₃R₆, and FITC-GA-MTS-H₃R₉) were provided by Anygen (Gwangju, Korea). The characterization of all the synthesized peptides is shown

in Supplementary Table 1.

Cell toxicity assay

AC16 human cardiomyocyte (1.2×10^4) cell line was seeded onto 96-well plates and incubated in Dulbecco's Modified Eagle's medium containing 10% fetal bovine serum for 16 h at 37°C. The cells were treated with different concentrations of GA, MTS, MTS-H₃R₆, MTS-H₃R₉, GA-MTS-H₃R₆, and GA-MTS-H₃R₉ and further cultured for 24 h. The cell viability using all drugs was conducted using the EZ-Cytox assay according to a protocol described previously [20].

Intracellular localization

To examine the subcellular localization of each peptide, AC16 (5×10^3) cells were seeded onto an 8-well chamber slide (Ibidi, Munich, Germany). Cells were then incubated for 24 h with 1 μ M of FITC-labeled GA, MTS, MTS-H₃R₆, MTS-H₃R₉, GA-MTS-H₃R₆, and GA-MTS-H₃R₉. The cells were treated with 50 nM MitoTracker Red for mitochondria or 100 nM LysoTracker for lysosomes for at least 15 min at 37°C. Cell nuclei were stained with Hoechst dye for 10 min and each sample was analyzed using a confocal microscope (Carl Zeiss, Jena, Germany).

Intracellular uptake and internalization

AC16 (1.5×10^5) cells were seeded onto six well plates. The cells were incubated for 16 h with 3.125 and 6.25 μ M of each peptide (with or without FITC-label). To quantitatively analyze the fluorescence intensity, intracellular uptake and internalization of the samples were performed by flow cytometry (BD Bioscience, San Jose, CA, USA).

Analysis of mitochondrial membrane potential

To determine the mitochondrial membrane potential (MMP) of peptides, JC-1 kit (Thermo Fisher Scientific, Grand Island, NY, USA) was used. AC16 (1.5×10^5) cells were seeded onto six well plates. The cells were treated with 6.25 μ M of each peptide for 16 h and then incubated with medium containing 10 μ g/ml JC-1 solution for 10 min at 37°C. The cells were then analyzed by flow cytometry.

Analysis of intracellular glutathione

Glutathione (GSH) was estimated using a Glutathione Colorimetric Assay Kit (BioVision, Milpitas, CA, USA). AC16 (1×10^6) cells were incubated for 16 h with 6.25 μ M of each peptide. GSH levels were measured by following a detailed protocol described previously [20].

Annexin V staining

Apoptosis was evaluated using a FITC Annexin V Apoptosis Detection Kit (BD Bioscience). AC16 (1×10^6) cells were incubated for 24 h with 6.25 μM of each peptide and the assay was performed according to the method previously described by the authors [21].

Cell viability assay

Cellular viability using the peptides was measured using a live/dead assay Kit (Invitrogen). Live cells were stained with green fluorescence by intracellular esterase enzyme activity, while dead cells were stained with red fluorescence in which ethidium homodimer-1 penetrates the plasma membrane thus damaging the cell integrity. AC16 (5×10^3) cells were incubated with 6.25 μM of each peptide for 24 h. The samples were then incubated with 1 μM of calcein acetoxyethyl ester and 4 μM of EthD-1 dissolved in PBS at 37°C for 30 min. The cells were imaged using a confocal microscope (Carl Zeiss).

Mitochondrial superoxide generation

Mitochondria ROS generation in the presence of the peptides were determined using MitoSox Red (Invitrogen). AC16 (5×10^3) cells were seeded onto an 8-well chamber slide (Ibidi). Cells were then incubated with 6.25 μM of each peptide for 16 h. The cells were treated with 5 mM MitoSox Red (Invitrogen) at 37°C for 30 min. The samples were imaged using a confocal laser microscope (Carl Zeiss).

Caspase-3 activity

Caspase-3 activity (BioVision) was determined following the manufacturer's instructions. AC16 (1×10^6) cells were incubated with 6.25 μM of each peptide for 24 h. Caspase-3 activity was determined as previously described by the authors [21].

Statistical analysis

Data were analyzed using GraphPad Prism software. Statistically significant differences were performed using the unpaired Student's *t*-test method.

RESULTS

Characteristics of GA-conjugated MTS-H₃R₉

The schematic depiction of the targeting activity of GA-MTS-H₃R₉ to mitochondria *via* endo-lysosomal pathway and its continuing activity as a cytoprotective agent is shown in Fig. 1.

Cytotoxicity

Cytotoxicity can be produced by environment conditions that induces cellular stress by means of intrinsic or extrinsic stimuli [22]. The effect of the MTS peptides on the viability was assessed using WST-1 and lactate dehydrogenase (LDH) cytotoxicity assay [23]. AC16 cells were exposed to GA, MTS, MTS-H₃R₆, MTS-H₃R₉, GA-MTS-H₃R₆, and GA-MTS-H₃R₉ at varying doses and exposure time. GA and MTS resulted in higher cell viability

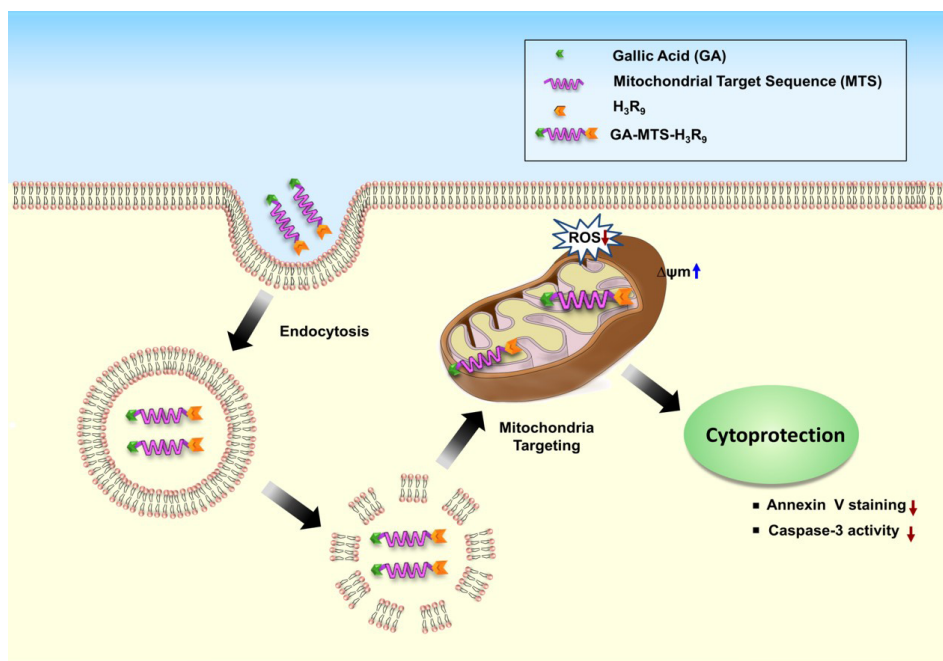


Fig. 1. Schematic description for the localization of the GA-MTS-H₃R₉ inside cells and its cytoprotective effect.

even when the peptide concentrations were increased (Fig. 2A). In contrast, MTS-H₃R₉-exposed cells showed more cytotoxicity than MTS-H₃R₆-exposed cells. Interestingly, MTS-H₃R₆ and MTS-H₃R₉ showed more cytotoxicity than GA-MTS-H₃R₆ and GA-MTS-H₃R₉. Also, we performed a LDH release assay. AC16 cells were treated similarly as in the EZ-Cytox cell viability assay. LDH release was higher in AC16 cells exposed to MTS-H₃R₉ than that with MTS-H₃R₆ depending on the dosage of peptides (Fig. 2B). Also, GA and MTS showed a low LDH release even when the peptide dosage were increased. However, membrane damage in MTS-H₃R₉-exposed cells was alleviated by GA-MTS-H₃R₉ treatment.

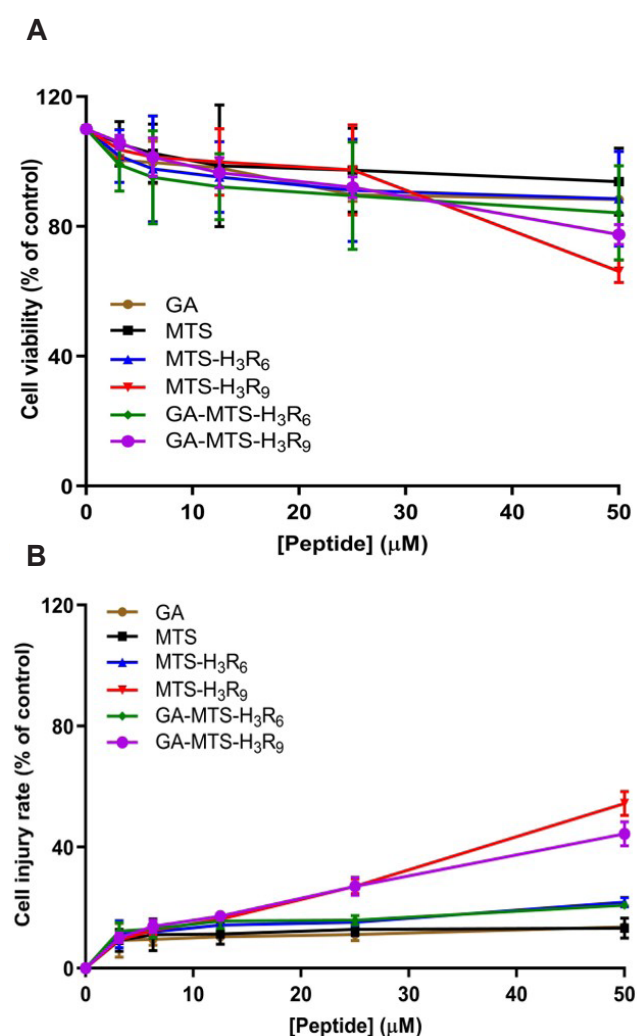


Fig. 2. Cell viability assay with GA-MTS-H₃R₉. Cell viability was determined using WST-1 and lactate dehydrogenase (LDH) assay. AC16 cells were exposed to different concentrations of GA, MTS, MTS-H₃R₆, MTS-H₃R₉, GA-MTS-H₃R₆, and GA-MTS-H₃R₉ up to 50 μM for 24 h. Panel (A) presents the results of the WST-1 assay and Panel (B) presents the results of LDH assay. GA, gallic acid; MTS, mitochondria targeting sequence. Data are indicated as the mean ± SD (n = 3).

Membrane internalization

To examine the membrane permeability activity of the peptides, we used propidium iodide (PI), a fluorescent indicator that stains prokaryotic or eukaryotic cells with damaged membranes based on membrane integrity [24]. We exposed AC16 cells with 3.125 and 6.25 μM of GA, MTS, MTS-H₃R₆, MTS-H₃R₉, GA-MTS-H₃R₆, and GA-MTS-H₃R₉ and cultured them for 16 h. With increasing doses, MTS-H₃R₉ exposed cells had increased PI staining compared to the untreated cells (Fig. 3A, B). GA-MTS-H₃R₉ exposed cells alleviated cellular damage compared to that of the MTS-H₃R₉ treatment.

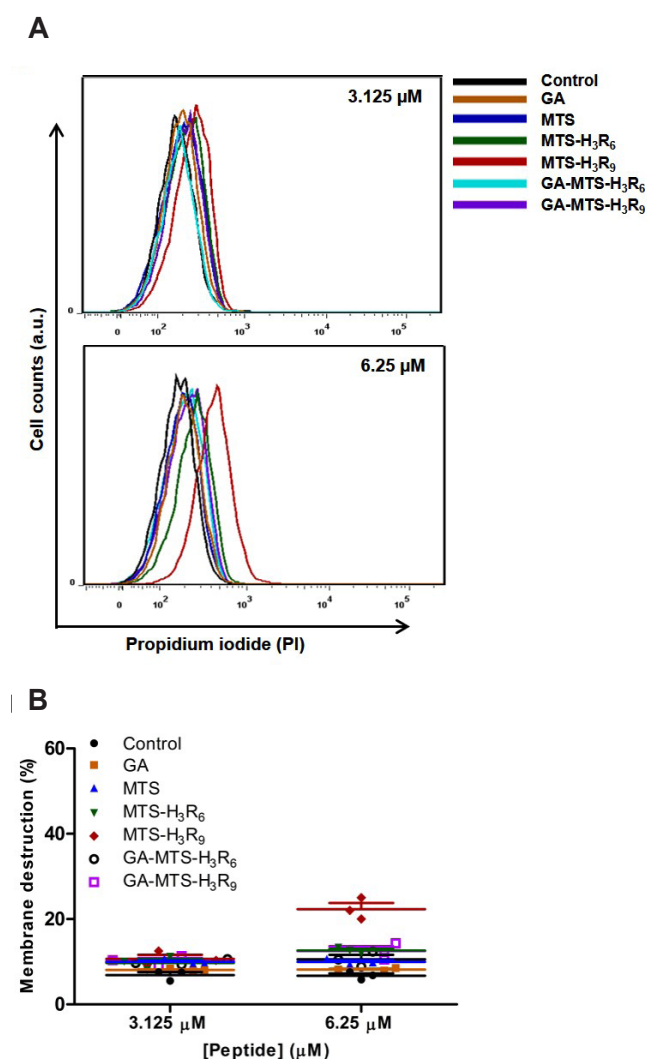


Fig. 3. Membrane internalization by GA-MTS-H₃R₉. AC16 cells were treated with 3.125 and 6.25 μM of GA, MTS, MTS-H₃R₆, MTS-H₃R₉, GA-MTS-H₃R₆, and GA-MTS-H₃R₉ for 16 h and were subsequently incubated with 5 μl of PI for 20 min. (A) PI internalization using flow cytometry analysis. (B) Quantitative graph showing each peptide from the PI fluorescence data shown in panel (A). GA, gallic acid; MTS, mitochondria targeting sequence; PI, propidium iodide.

Intracellular uptake

To investigate cellular uptake process of the peptides, AC16 cells were treated with 3.125 and 6.25 μM of FITC-GA, FITC-MTS, FITC-MTS-H₃R₆, FITC-MTS-H₃R₉, GA-MTS-H₃R₆, and GA-MTS-H₃R₉ to assess doses dependent activity of these peptides. Interestingly, when the dose of the peptides was increased, cellular uptake of all the FITC-labelled peptide showed increased activity compared with FITC-MTS except for GA (Fig. 4A, B). According to these results, a dose of 6.25 μM was used for the subsequent experiments.

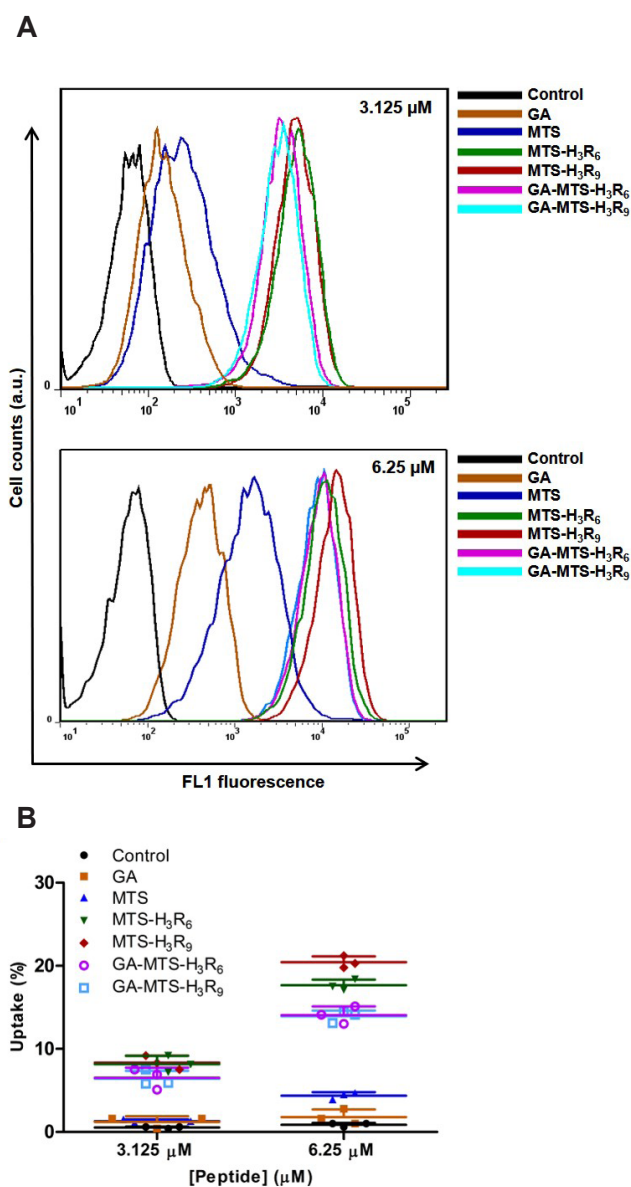


Fig. 4. Intracellular uptake of GA-MTS-H₃R₉. AC16 cells were treated with 3.125 and 6.25 μM of FITC-labelled GA, MTS, MTS-H₃R₆, MTS-H₃R₉, GA-MTS-H₃R₆, and GA-MTS-H₃R₉ for 16 h. (A) Cellular uptake of each FITC-labelled peptide using flow cytometry analysis. (B) Quantitative graph showing each peptide from uptake data depicted in panel (A). GA, gallic acid; MTS, mitochondria targeting sequence.

Intracellular distribution

To examine intracellular distribution of the peptides, AC16 cells were treated with 1 μM of FITC-labelled peptides. After 16 h of incubation, the cells were stained with LysoTracker (red) and then visualized by confocal microscopy. Cells incubated with GA and MTS showed a distribution around lysosomes. In contrast, MTS-H₃R₆, MTS-H₃R₉, GA-MTS-H₃R₆, and GA-MTS-H₃R₉ displayed colocalization of LysoTracker (red) following endosomal release from the lysosomes into cytosol in the cells due to escape from the endo-lysosome after cellular uptake (Fig. 5).

Intracellular mitochondrial targeting

To evaluate mitochondrial-targeting ability of the peptides using confocal microscopy, AC16 cells were treated with 1 μM of FITC-GA, FITC-MTS, FITC-MTS-H₃R₆, FITC-MTS-H₃R₉, FITC-GA-MTS-H₃R₆, and FITC-GA-MTS-H₃R₉ for 24 h. Cells incubated with FITC-MTS-H₃R₆ and FITC-MTS-H₃R₉ with and without GA exhibited colocalization with MitoTracker in the mitochondria of the AC16 cells as compared to MTS treated cells (Fig. 6). Particularly, FITC-MTS-H₃R₉ and FITC-GA-MTS-H₃R₉ showed the highest targeting of mitochondria in the cells.

In addition, cell cytotoxicity was examined under similar conditions including the mitochondria targeting experiments. AC16 cells were exposed to each peptide for 24 h. For this assay, the cytotoxicity of the peptides was investigated using WST-1 and LDH release assay. Also, we performed a live/dead viability assay. Regardless of the dose of each peptide, all peptides conferred high cell viability (Supplementary Fig. 1).

Reactive oxygen species

To evaluate the level of ROS generated by cells exposed to these peptides, AC16 cells were treated with 6.25 μM of each peptide for 12 h. We examined the levels of GSH, as hydrophilic antioxidant enzymes act against ROS production for protection of cells [25]. As shown in Fig. 7A, decreased GSH were observed in the cells exposed to MTS-H₃R₉ in the grafting of GA in MTS-H₃R₉, compared to MTS treatment.

To further investigate whether MTS-H₃R₉ induces ROS production when each peptide was delivered into the mitochondria, we employed Mitosox Red, as an indicator of mitochondria derived ROS production [26]. As shown in Fig. 7B, compared to control cells, GA-MTS-H₃R₉ showed a weak red fluorescence signal compared to that of MTS-H₃R₉ via superoxide production. These results suggest that the conjugation of antioxidant GA peptide to the MTS-H₃R₉ and GA-MTS-H₃R₉ may rescue cells against oxidative stress by enhancing antioxidant activity in the cells due to the polyphenol compound related to the free hydroxyl groups on the aromatic ring in GA.

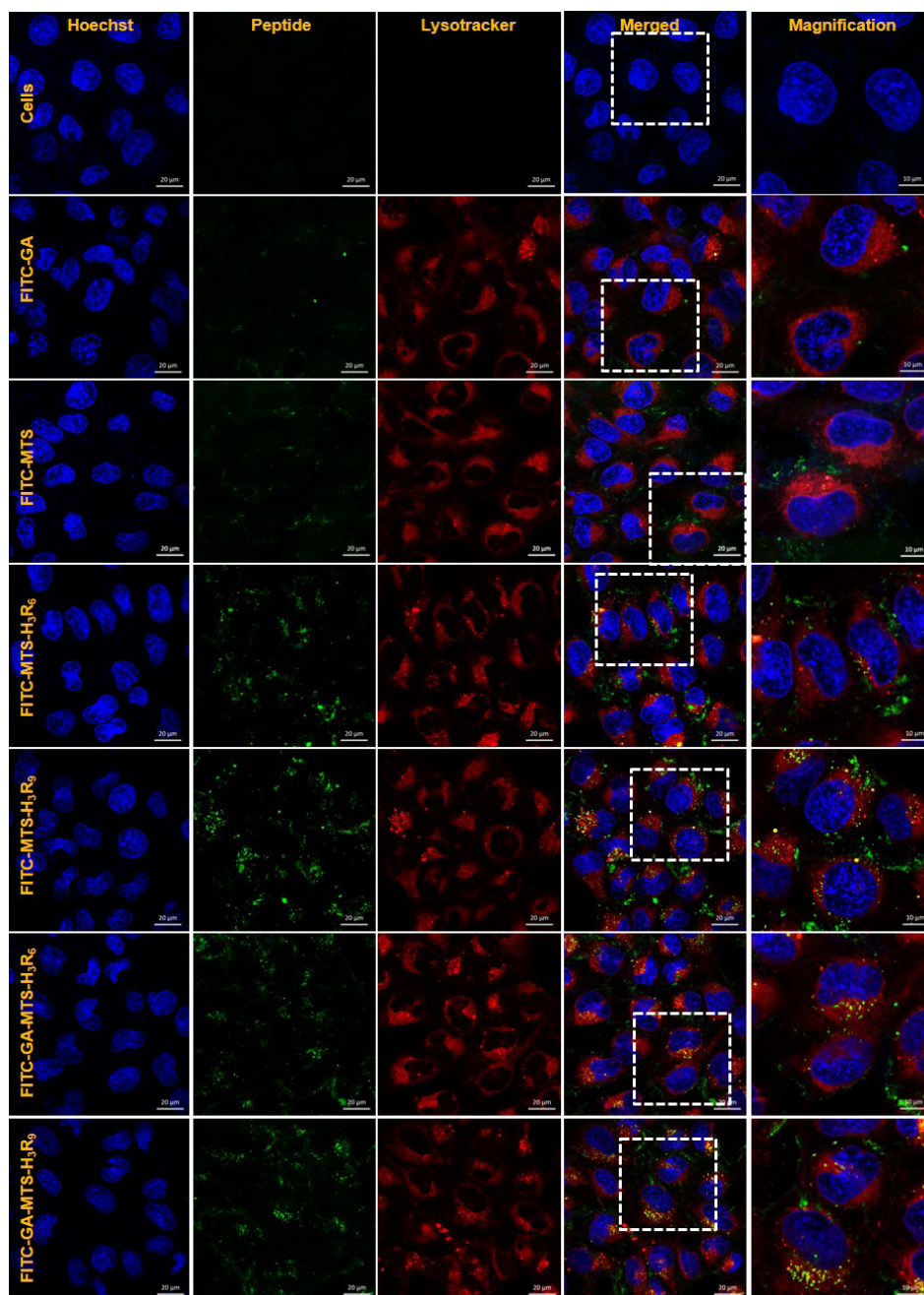


Fig. 5. Intracellular distribution of GA-MTS-H₃R₉. AC16 cells were treated with 1 μ M of FITC-labeled GA, MTS, MTS-H₃R₆, MTS-H₃R₉, GA-MTS-H₃R₆, and GA-MTS-H₃R₉ for 16 h. The cells were stained with LysoTracker (red) for 20 min and counterstained with Hoechst stain (blue) for 10 min and imaged by confocal microscopy. Scale bar is 10 μ m. GA, gallic acid; MTS, mitochondria targeting sequence.

Mitochondrial membrane potential

The change in MMP is an indicator of mitochondrial function which representing apoptotic process following cytochrome c release from mitochondria [27]. To investigate the mitochondrial depolarizing effect of GA-MTS-H₃R₉, we measured the MMP using JC-1 probe, a lipophilic cationic dye that exhibits a J-aggregation form with red fluorescence because of high MMP in healthy cells. However, the monomeric form exhibits green fluorescence in low MMP by depolarization of the mitochondria in apoptotic cells [28]. AC16 cells treated with 6.25 μ M of all the peptides were examined using flow cytometry. When compared to untreated cells, treatment of AC16 cells with MTS-H₃R₆ and MTS-H₃R₉

induced MMP dissipation (19.0% and 43.5%, respectively). Interestingly, MTS-H₃R₉-treated cells showed significantly greater MMP disruption compared to that in MTS-H₃R₆ treated cells. In addition, GA-MTS-H₃R₉ showed a significant reduction in MMP depolarization compared to that of MTS-H₃R₉ (43.5% and 21.8%, respectively) (Fig. 7C). Furthermore, ROS generation and MMP experiments were carried out employing the similar conditions as those used in the mitochondria targeting assays. We examined GSH and JC-1 assay and all peptides used in this experiment did not trigger cellular damage and the cells adapted with peptide exposure at a low dose (Supplementary Fig. 2).

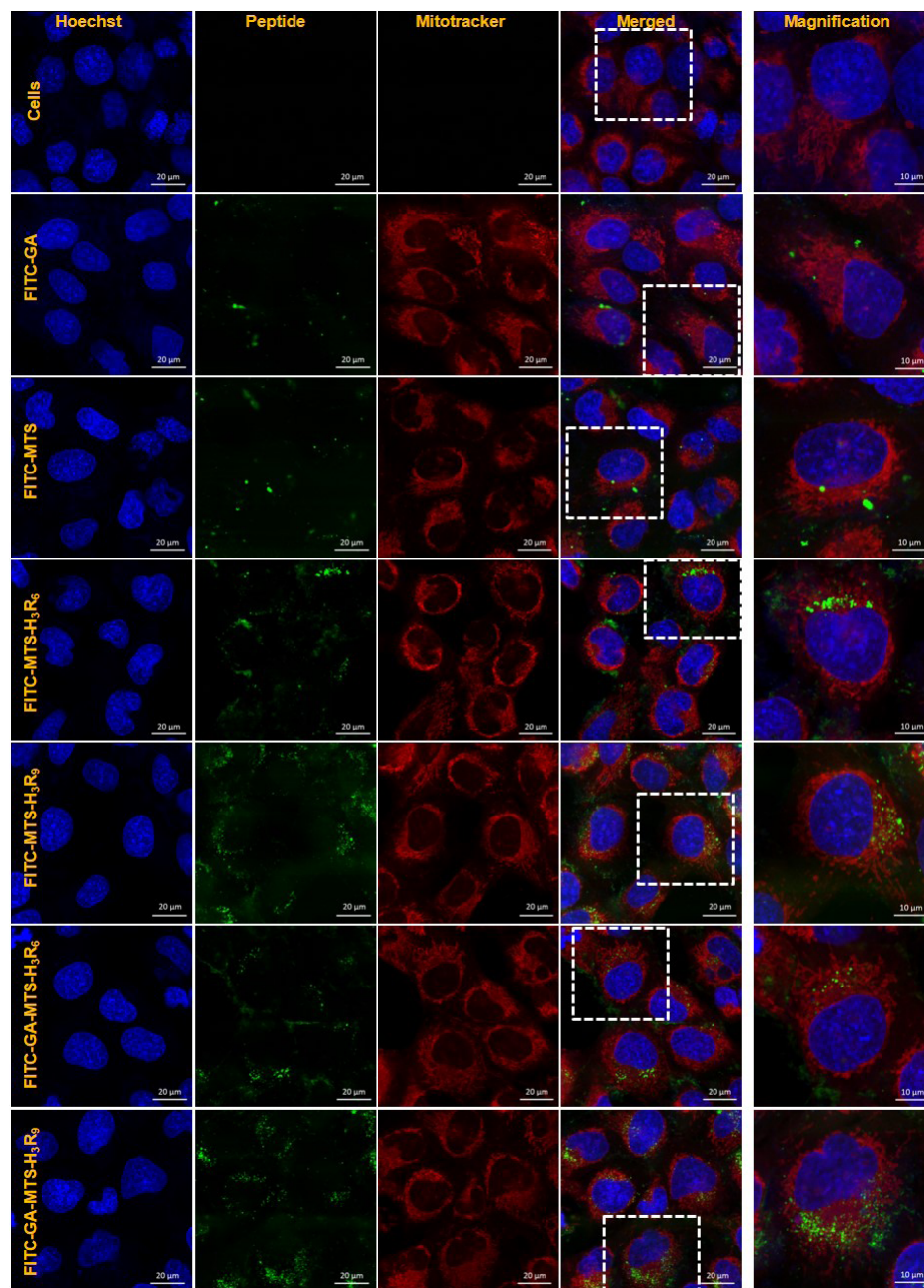


Fig. 6. Intracellular mitochondria localization of GA-MTS-H₃R₉. AC16 cells were exposed to 1 μ M of FITC-labeled GA, MTS, MTS-H₃R₆, MTS-H₃R₉, GA-MTS-H₃R₆, and GA-MTS-H₃R₉ for 24 h. The cells were stained with MitoTracker (red) for 20 min and counterstained with Hoechst stain (blue) and then imaged by confocal laser scanning microscopy. Scale bar is 20 μ m. GA, gallic acid; MTS, mitochondria targeting sequence.

Apoptosis

We evaluated the apoptotic activity of GA-MTS-H₃R₉ by FACS analysis using an Annexin V/PI double staining. Annexin V binds to a surface exposed to phosphatidyl serine from the plasma membrane during apoptotic processes resulting in the disruption of plasma membrane. In addition, PI passes through the plasma membrane and binds to cellular DNA of necrotic cells [21,24]. AC16 cells were treated with 6.25 μ M of each peptide for 24 h. As shown in Fig. 8A, MTS-H₃R₆ and MTS-H₃R₉ showed an increase in late apoptosis (17.4% and 26.7%, respectively) compared to that in control group cells (4.5%). Interestingly, AC16 cells exposed to GA-MTS-H₃R₉ exhibited a decrease in late apoptotic cells

(18.5%) than those exposed to MTS-H₃R₉ (26.7%). Furthermore, experiments related to apoptosis were conducted under the similar conditions similar to the mitochondria targeting assays. We examined annexin V/PI staining. We observed that exposure to all the peptides did not result in cell death in these conditions (Supplementary Fig. 3).

To further confirm the anti-apoptotic activity of GA-MTS-H₃R₉, we applied the caspase-3 activity as executioner caspases that was mediated *via* intrinsic apoptosis pathway due to various cellular stresses [29]. We performed these experiments using similar conditions as those of the annexin V/PI staining. Compared with MTS peptides, GA-MTS-H₃R₉-exposed AC16 cells displayed decreased caspase-3 activity compared to MTS-H₃R₉ treated cells (Fig. 8B).

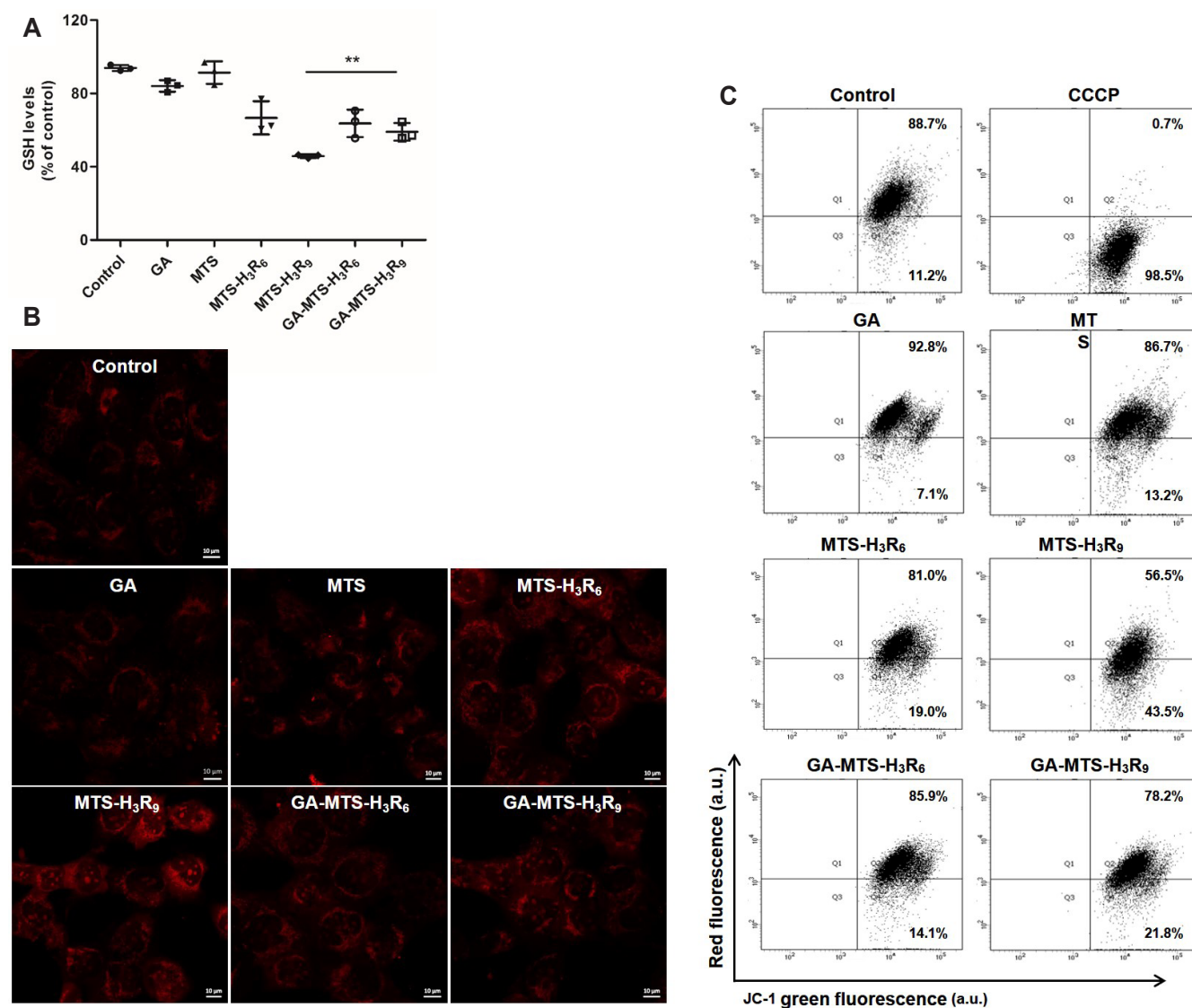


Fig. 7. ROS levels and mitochondrial membrane potential (MMP) with GA-MTS-H₃R₉. (A) AC16 cells were exposed to 6.25 μ M of GA, MTS, MTS-H₃R₆, MTS-H₃R₉, GA-MTS-H₃R₆, and GA-MTS-H₃R₉ for 16 h. Intracellular ROS levels were assessed by GSH assay. (B) AC16 cells were treated using the same conditions as those panel (A). The cells were treated with 20 μ M MitoSox for 16 h. Cells were imaged by confocal microscopy. Scale bar is 10 μ m. (C) AC16 cells were treated using the same conditions as those in panel (A). The cells were added to 2 μ M JC-1 and then incubated for 20 min. MMP was determined by flow cytometry analysis. Data are indicated as the mean \pm SD (n = 3). ROS, reactive oxygen species; GA, gallic acid; GSH, glutathione; MTS, mitochondria targeting sequence; CCCP, carbonyl cyanide 3-chlorophenylhydrazine. **p < 0.01.

DISCUSSION

In our study, we demonstrated that MTS-H₃R₉ conjugated to GA (GA-MTS-H₃R₉) regulated mitochondrial apoptosis by employing dual functional strategies such as by acting as a delivery vehicle and by functioning as a therapeutic agent. Previous reports suggested that cell penetrating peptide (CPP) such as cationic, hydrophobic, and amphipathic peptides comprised of 5–20 amino acids traverse the tissue and cell membranes to biologically deliver active cargo into cells. Among the CPPs, arginine-rich peptides with highly positive charges show characteristics demonstrating penetration of plasma membranes [16]. In our study, we indicated that with or without GA in FITC-MTS-H₃R₆

and FITC-MTS-H₃R₉, the cationic peptide-modified MTS may facilitate cell membrane penetration caused by cell penetrating peptides with arginine residues and then subsequently improving cellular uptake, potentially optimizing subcellular targeting of the mitochondria.

Subcellular localization into the cells for cellular targeting via endocytosis is a major factor for successful drug delivery [30]. In our study, we showed that MTS-H₃R₆ and MTS-H₃R₉, with or without GA has the potential to surmount endosomal/lysosomal hurdles to achieve mitochondria-targeting via the endocytosis system (Fig. 5).

The results demonstrated that cells exposed to GA-MTS-H₃R₉ showed high cell viability against mitochondrial-mediated cell

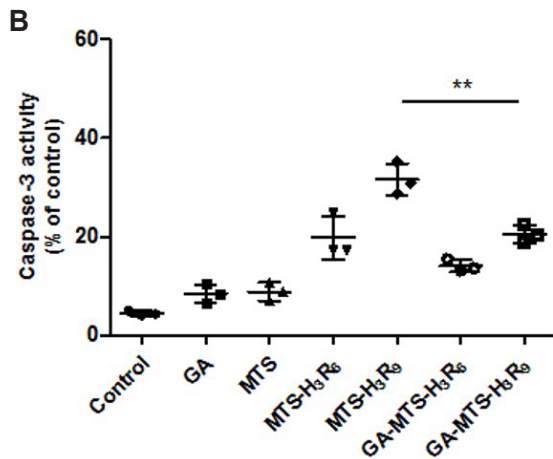
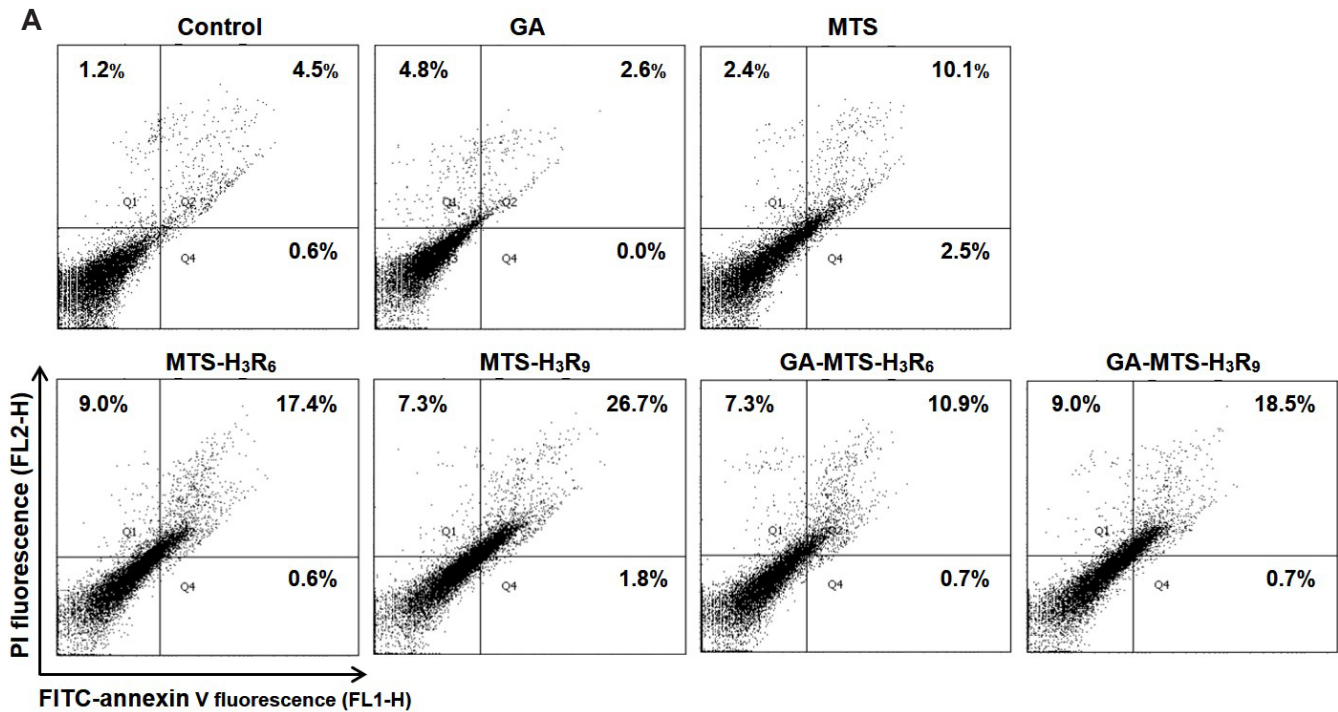


Fig. 8. Induction of cytoprotection by GA-MTS-H₃R₉. (A) Anticancer activity of each peptide. AC16 cells were exposed to 6.25 μ M of GA, MTS, MTS-H₃R₆, MTS-H₃R₉, GA-MTS-H₃R₆, and GA-MTS-H₃R₉ for 24 h. Apoptosis levels for each group were measured by flow cytometry analysis after annexin V staining. Q1 indicates necrotic cells, Q2 indicates late apoptotic cells, Q3 indicates intact cells, and Q4 indicates early apoptotic cells. (B) Caspase-3 activity of each peptide. AC16 cells were treated using the same conditions as those in panel (A). Caspase-3 activity was assessed as described in Methods. Data are indicated as the mean \pm SD (n = 3). GA, gallic acid; MTS, mitochondria targeting sequence. **p < 0.01.

death induced by increased ROS generation, disrupted mitochondrial membrane potential, and anticancer activity. However, GA-MTS-H₃R₉ showed reduced cytotoxicity and mitochondria-triggered cell damage compared with MTS-H₃R₉ at 6.25 μ M dose. Furthermore, GA-MTS-H₃R₉ alleviated intrinsic cell death in AC16 cells caused by ROS generation, disrupted mitochondrial membrane potential, and induced cell death at the 6.25 μ M dose compared with that by MTS-H₃R₉ treatment. Our results indicate that GA-MTS-H₃R₉ as a targeted mitochondria-selective vehicle displayed cytoprotective effects against cellular damage. It is established that mitochondria-targeted GA-MTS-H₃R₉ can be used as a bifunctional peptide that provides efficient mitochondrial target carrier as well as function as an effective therapeutic agent. These findings suggest that this peptide vehicle can be employed for therapeutic applications for mitochondria-associated disorders.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary data including one table and three figures can be found with this article online at <https://doi.org/10.4196/kjpp.2022.26.1.15>.

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