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Novel self-micellizing anticancer lipid nanoparticles induce cell death of colorectal cancer cells

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ABSTRACT

In the present study, we developed a novel drug-like self-micellizing anticancer lipid (SMAL), and investigated its anticancer activity and effects on cell death pathways in human colorectal cancer (CRC) cell lines. Three self-assembled nanoparticles were prepared, namely, SMAL102 (lauramide derivative), SMAL104 (palmitamide derivative), and SMAL108 (stearamide derivative) by a thin-film hydration technique, and were characterized for physicochemical and biological parameters. SMAL102 were nanosized (160.23 ± 8.11 nm) with uniform spherical shape, while SMAL104 and SMAL108 did not form spherical shape but formed large size nanoparticles and irregular in shape. Importantly, SMAL102 showed a cytotoxic effect towards CRC cell lines (HCT116 and HT-29), and less toxicity to a normal colon fibroblast cell line (CCD-18Co). Conversely, SMAL104 and SMAL108 did not have an anti-proliferative effect on CRC cell lines. SMAL102 nanoparticles were actively taken up by CRC cell lines, localized in the cell membrane, and exhibited remarkable cytotoxicity in a concentration-dependent manner. The normal colon cell line showed significantly less cellular uptake and non-cytotoxicity as compared with the CRC cell lines. SMAL102 nanoparticles induced caspase-3, caspase-9, and PARP cleavage in HT-29 cells, indicating the induction of apoptosis; whereas LC3B was activated in HCT116 cells, indicating autophagy-induced cell death. Collectively, these results demonstrate that SMAL102 induced cell death via activation of apoptosis and autophagy in CRC cell lines. The present study could be a pioneer for further preclinical and clinical development of such compounds.

1. Introduction

Great efforts have been made in cancer therapy due to the high heterogeneity and adaptations of tumor cells [1]. The high mortality rate of cancer is mainly attributed to multidrug resistance, where chemotherapy plays an important role in treatment and has become one of the most widely used tools in combating cancer [2]. However, administration of free anticancer drugs has often resulted in a poor therapeutic response due to a short half-life in blood circulation, poor tumor specificity, and accumulation in normal cells [3]. In order to overcome these limitations, various drug delivery systems have been exercised. Specifically, nano-sized carriers have gained tremendous attention in tumor drug delivery. Growing interests in anticancer drug delivery systems suggest that the use of such nanocarriers can enhance the therapeutic efficacy of chemotherapeutic drugs with a reduced risk of adverse effects. The biocompatibility (non-toxicity) of these drugs to normal cells would be additional advantage [4–6]. Currently, greater efforts have been made to identify and design efficacious nanocarriers in cancer targeting. Any nanomaterial that can enhance the chemotherapeutic response of a drug moiety while being non-toxic to normal cells is highly desired, especially a pro-apoptotic agent [7,8].

A pro-apoptotic agent has preferential selectivity for inducing apoptosis in cancer cells [9]. Pro-apoptotic agents like synthetic sphingolipid plays an important role in cell differentiation, cell cycle arrest, apoptosis, growth inhibition and senescence [10,11]. The bioactive sphingolipid acts as a biochemical and biophysical mediator of cell growth arrest and/or cellular apoptosis. In *in vivo* conditions, sphingomyelinases produce a pro-apoptotic and anti-mitogenic second messenger regulating multiple signaling cascades leading to cell growth arrest and/or apoptosis in various cell types [12,13]. Therefore, the use of pro-apoptotic agents

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may enhance the efficacy of existing treatments without further exacerbating toxicities.

Nanoscale packages have drawn considerable attention for their effective drug delivery strategies to treat cancer [14,15]. Advances in self-assembled based drug delivery systems have had a great impact on modern medicine, particularly cancer chemotherapy [16]. In particular, self-assembled nanoparticles have been widely investigated for their ability to load a range of poorly water-soluble anticancer drugs and to passively accumulate in leaky tumor sites [17,18].

In the present study, we thoroughly investigated the physicochemical properties and biological response of a novel nanomaterial. Accounting reported issues, we designed a novel pro-apoptotic nanocarrier having preferential selectivity towards cancer cells. We developed a drug-like self-micellizing anticancer lipid (SMAL) and investigated its self-assembling properties and thermodynamic stability. The single molecules SMAL-based self-assembled nanoparticles were named as a SMAL102, SMAL104, and SAML108. The anticancer effect of SMAL-based nanoparticles (SMAL 102, 104, and 108) was studied in normal as well as colorectal cancer cells (CRC) and a detailed characterization of cell death pathways was performed. Based on cytotoxicity assay, SMAL102 micelle was selected for detailed study. Our unique findings suggest that SMAL102 exerts putative anticancer effects through modulation of apoptosis and autophagy pathways. To the best of our knowledge, this is the first report examining the anticancer properties of SMAL102.

2. Materials and methods

2.1. Materials

1,3-bis(*N*-2-(hydroxyethyl) lauroyl amino)-2-hydroxy propane (SMAL102), 1, 3-bis (*N*-2-(hydroxyethyl) palmitoyl amino)-2-hydroxy propane (SMAL104) and 1, 3-bis (*N*-2-(hydroxyethyl) tetramide amino)-2-hydroxy propane (SMAL108) were purchased from Macrocare Tech., LTD, Ochang, South Korea). Primary antibodies (Poly(ADP-ribose) polymerase-1, caspase-3, caspase-9, LC3B, and GAPDH) and the corresponding secondary antibodies for western blotting were purchased from Cell Signaling Technology (Danvers, Massachusetts, USA). Propidium iodide, ribonuclease, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma–Aldrich (St. Louis, MO, USA). 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) (ammonium salt) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). All other chemicals were of reagent grade and used without further purification.

2.2. Preparation of nanoparticles

Nanoparticles were prepared using the thin-film hydration method. Briefly, SMAL102, SMAL104 and SMAL108 were dissolved in chloroform and methanol (2:1), and dried with a vacuum rotary evaporator to form a thin film. The film was hydrated by the addition of distilled water, heated to 60 °C for 1 h in a sealed container, and subjected to vortex mixing for 1 min. The hydrated thin film was stored in the refrigerator at 4 °C overnight, followed by sonication for 10 min, and subsequent storage at 4 °C until use. For the cellular uptake study, SMAL102 was prepared using rhodamine fluorescent dye.

2.3. Measurement of nanoparticle size, surface charge, and morphology

The hydrodynamic size, polydispersity index (PDI), and zeta potential was determined using a high performance dynamic light scattering (DLS) device with Malvern Zetasizer Nano ZS (Malvern Instrument, UK) at a fixed angle of 90°. The SMAL102, SMAL104, and SMAL108 was sonicated for 30 s and then diluted in distilled water for the experiment. Particle size, PDI, and surface zeta potential were measured at 25 °C. All measurements were performed in triplicate. The morphology of SMAL102, SMAL104, and SMAL108 nanoparticle was examined by transmission electron microscope (TEM; H7600, Hitachi, Tokyo, Japan) at an accelerating voltage of 100 kV. A drop of nanoparticle dispersion was deposited on the copper grid and counterstained by 2% phosphotungstic acid prior to TEM imaging.

2.4. Stability studies

The stability of the SMAL102 nanoparticle was assessed by DLS after 1, 5, 10, 15 and 20 days of storage in the refrigerator (4 °C) or at ambient room temperature (25 °C).

2.5. Cell culture

All the cell lines were purchased from American Type Culture Collection (Manassa, Va, USA). The normal human colon fibroblast cell line (CCD-18Co) was grown in DMEM medium and the human colorectal cancer cell lines (HCT116 and HT-29) were grown in RPMI1640 medium. Medium was supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were maintained at 37 °C with 5% CO_2 in a humidified incubator.

2.6. Cytotoxicity assay

The cytotoxicity of DMSO-based SMA102 and the three different nanoparticles were assessed by MTT assay. Briefly, cells were seeded in 96-well plates (5000 cells/well) and allowed to grow for 24 h. The cells were treated with various concentrations of nanoparticle and DMSO-based SMAL102 and were incubated for 48 h. The medium containing the drug was removed and 100 μL MTT solution (1 mg/mL) was added to each well. The cells were incubated for 2 h at 37 °C in a humidified environment of 5% atmospheric CO_2. MTT solution was removed from wells, the formazan crystals formed were dissolved in 100 μl DMSO, and the absorbance was measured at 570 nm using a microplate reader.

2.7. Colony formation assay

HCT116 and HT-29 cells were harvested from logphase of growth and plated at densities of 100–200 cells per well in 6-well plates. After 24 h, the cells were treated with SMAL102 or drug vehicle. Every 3 days media were changed with drug. After 10–14 days in culture, the media was removed, and adherent colonies were fixed with methanol and stained with hematoxylin and eosin. The plates were photographed with a digital camera and the colonies was counted and plotted in a bar graph.

2.8. Cellular uptake study

CCD-18Co, HCT116 and HT-29 (2×10^3 cells) were seeded on 35 mm dishes and incubated for 24 h at 37 °C. Cells were then incubated with 100 μg/mL SMAL102-rhodamine for 2 h followed by rinsing three times with cold PBS. Cells were then fixed with 4% paraformaldehyde in PBS at room temperature for 3 min, washed twice with PBS, and sealed with mounting media containing DAPI (Vectashield, Vector Laboratories, Burlingame, CA, USA). Images were captured using confocal laser scanning microscopy (Nikon A1+, Japan).

For quantitative analysis, 2×10^5 cells were seeded on 35 mm dishes and grown to 90% confluence under cell culture condi-