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The α-tocopherol derivative ESeroS-GS induces cell death and inhibits cell motility of breast cancer cells through the regulation of energy metabolism

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ABSTRACT

Cancer cells are known to exhibit different hallmarks compared with normal cells. Therefore, targeting these features may improve the response to cancer therapy. In this study, we provided direct evidence that the α-tocopherol derivative ESeroS-GS inhibited the viability, migration, and invasion of breast cancer cells. ESeroS-GS induced cell death in different cancer cells in a dose-dependent manner but showed no significant effects on MCF-10A mammary epithelial cells. Although the ESeroS-GS-induced cell death in MDA-MB-231 breast cancer cells was accompanied with the generation of reactive oxygen species and the down regulation of mitochondrial membrane potential (MMP), no such effect on reactive oxygen species and MMP was seen in MCF-10A cells. Further studies indicated that ESeroS-GS down-regulated the expression of hexokinase II, SDH B, UQCRC2 and COX II in MDA-MB-231 cells but not in MCF-10A cells. The down-regulation of these enzymes accounts for the decreased oxidative phosphorylation (OXPHOS) and glycolysis in MDA-MB-231cells upon ESeroS-GS treatment. We also found that sub-toxic concentration of ESeroS-GS treatment resulted in the impairment of F-actin cytoskeleton assembly and the consequently decreased migratory and invasive ability of MDA-MB-231 cells, which might be due to the inhibition of cellular energy metabolism. These results indicate that ESeroS-GS shows potential to become a novel anti-cancer agent by targeting the energy metabolism of cancer cells.

1. Introduction

Cancer is a key public health threat worldwide, and breast cancer is the most frequently diagnosed cancer and the leading cause or second leading cause of cancer death among females (DeSantis et al., 2014). More than one million new cases of breast cancer are diagnosed worldwide each year (Rana et al., 2013). In China, the incidence of breast cancer also had increased at an alarming rate over the past two decades (Huang et al., 2014).

Cancer cells, unlike normal cells, divide and grow uncontrollably, forming malignant tumors. They have six features: sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. Recently, the energy metabolism of cancer cells was investigated as an additional hallmark (Hanahan and Weinberg, 2011). The metabolic fluxes in cancer cells are different from those found in normal cells. Tumor

Abbreviations: ATP5A, ATP synthase subunit alpha; COX II, cytochrome c oxidase subunit II; ECAR, extracellular acidification rate; EPC-K1, L-ascorbic acid 2-[3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethytridecyl)-2H-1-benzopyran-6-yl-hydrogen phosphate] potassium salt; ESeroS-GS, γ-L-glutamyl-S-[2-[[[3,4- dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2H-1-benzopyran-6-yl]oxy] carbonyl]-3-[[2-(1H-indol-3-yl) ethyl] amino]-3-oxopropyl]-L-cysteinylglycine sodium salt; FCCP, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone; MMP, mitochondrial membrane potential; OCR, oxygen consumption rate; OXPHOS, oxidative phosphorylation; WST-8, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)- 5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt; 2-DG, 2-deoxyglucose; SDHB, succinate dehydrogenase complex, subunit B; UQCRC2, ubiquinol-cytochrome c reductase core protein II; DAPI, 4′,6-diamidino-2-phenylindole; TMRM, tetramethyl rhodamine methyl ester

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cells exhibit an increased rate of glycolysis as the fuel of cell growth and division, even in the presence of a high O_2 concentration in a phenomenon termed as the Warburg effect (Sotgia et al., 2011; Vander Heiden et al., 2009). However, mitochondrial function is also of crucial importance for cancer cells, particularly under low glucose conditions commonly observed in solid tumors (Birsoy et al., 2014; Zhang et al., 2014).

Targeting the oxidative phosphorylation (OXPHOS) and/or glycolysis of cancer cells might be a novel strategy for the therapy of cancer (Zhao et al., 2013b), and anticancer agents that specifically target the cancer cell mitochondria are denoted as 'mitocans'. This group of drugs is represented by redox-silent vitamin E analogs (Dong et al., 2009; Neuzil et al., 2007), including α–TOS (Neuzil et al., 2001), MitoVES (Dong et al., 2011a; Dong et al., 2011b), α-TEA (Dong et al., 2012), Mito-Q, Mito-CP (Cheng et al., 2012), and tocotrienols (Wong and Radhakrishnan, 2012).

In our previous investigations, we reported the antioxidant and anti-inflammatory effect of two novel vitamin E derivatives. γ-L-glutamyl-S-[2-[[[3,4-dihydro-2,5,7,8- tetramethyl-2-(4,8,12-trimethyltridecyl)-2H-1-benzopyran-6-yl]oxy]carbonyl]-3-[[2-(1H-indol-3-yl) ethyl] amino]-3-oxopropyl]-L-cysteinylglycine sodium salt (ESeroS-GS; Fig. 1A) contains α-tocopherol and glutathione on succinic acid as the core. L-ascorbic acid 2-[3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethytridecyl)- 2H-1-benzopyran-6-yl-hydrogen phosphate] potassium salt (EPC-K1; Fig. 1B) contains α-tocopherol and L-ascorbic acid moiety. Due to their hydrophobic group and hydrophilic group, both ESeroS-GS and EPC-K1 can form micelle spontaneously in water and can be used as water-soluble analogs of vitamin E. Previously, our work indicated that ESeroS-GS can act as an anti-inflammatory agent inhibiting the activation of astrocytes (Wei et al., 2003) or macrophages (Duan et al., 2011) by modulating NF-κB signaling via a lipid raft-dependent mechanism. We also find that EPC-K1 protects neuronal cells from oxidative stress by scavenging free radicals effectively (Wei et al., 1999a; Wei et al., 1999b). However, the potential anticancer role of ESeroS-GS and EPC-K1 had not been examined. Thus, in this study, we investigated the effects of ESeroS-GS and EPC-K1 on cancer cells, and the underlying mechanisms were explored.

2. Materials and methods

2.1. Materials

ESeroS-GS and EPC-K1 were obtained from Senju Pharmaceutical Co. Ltd. (Osaka, Japan). Hoechst 33342 and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, a water-soluble

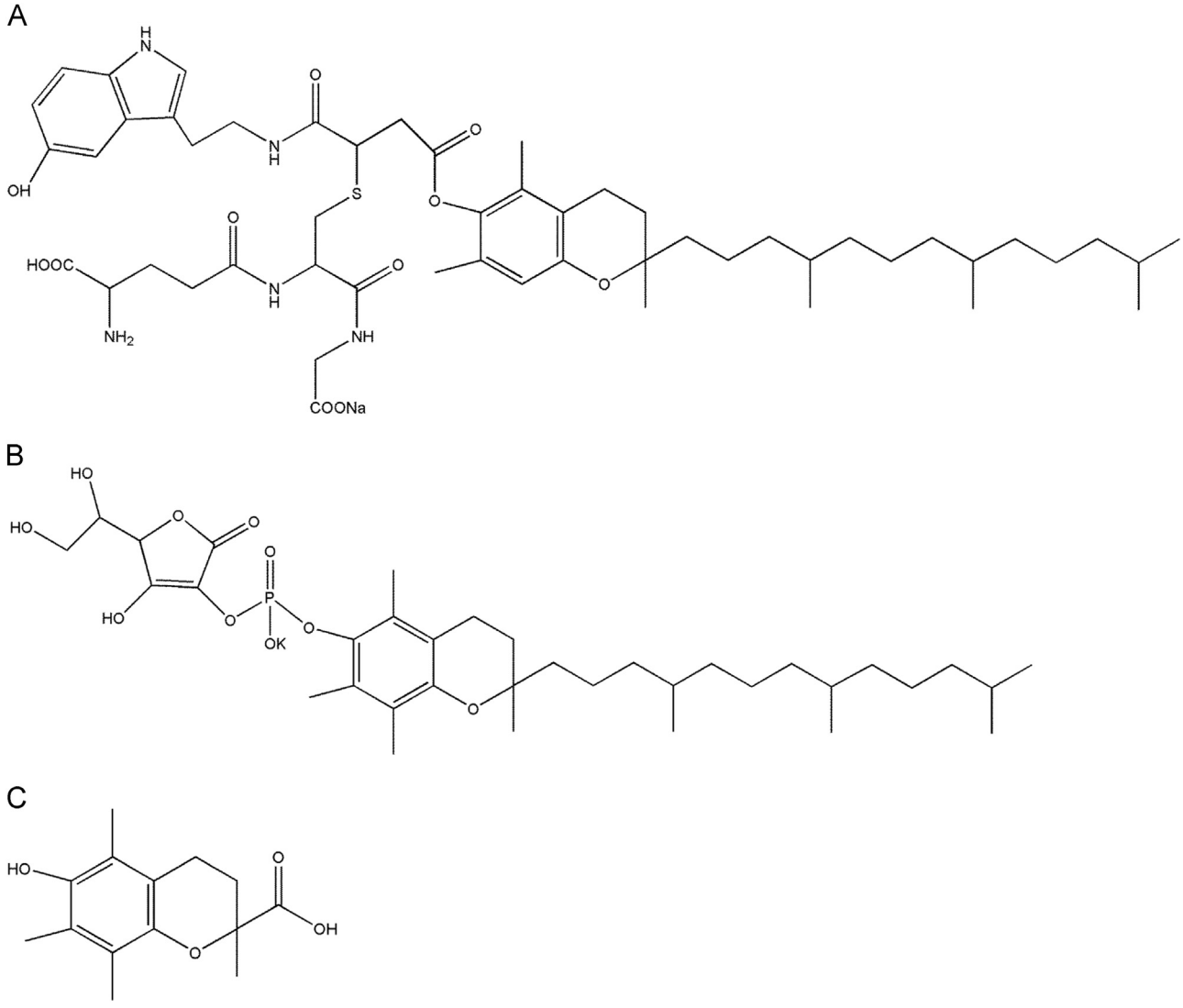

Fig. 1. Structure of ESeroS-GS (A), EPC-K1 (B) and Trolox (C).