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Solamargine inhibits migration and invasion of human hepatocellular carcinoma cells through down-regulation of matrix metalloproteinases 2 and 9 expression and activity

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ABSTRACT

Solamargine is a steroidal alkaloid glycoside isolated from *Solanum nigrum*. The aim of this study was to investigate the effects of solamargine on tumor migration and invasion in aggressive human hepatocellular carcinoma cells. The MTT assay was used to assess the effects of solamargine on the viability of HepG2 cells. Migration and invasion ability of HepG2 cells under solamargine treatment were examined by a wound healing migration assay and Boyden chamber assay, respectively. Western blotting assays were used to detect the expression of MMP-2 and MMP-9 proteins and MMP-2 and MMP-9 activity were analyzed by gelatin zymography assay. Solamargine reduced HepG2 cell viability in a concentration-dependent manner. At 7.5 μM solamargine decreased cell viability by less than 20% in HepG2 cells. A wound healing migration assay and Boyden chamber invasion assay showed that solamargine significantly inhibited in vitro migration and invasion of HepG2 cells. At the highest dose, solamargine decreased cell migration and invasion by more than 70% and 72% in HepG2 cells, respectively. Western blotting and gelatin zymography results showed that solamargine reduced expression and function of MMP-2 and MMP-9 proteins. In conclusion, the results showed that solamargine significantly inhibits migration and invasion of HepG2 cells by down-regulating MMP-2 and MMP-9 expression and activity.

1. Introduction

The lethal outcome of all cancers is due to the spreading of primary tumor cells and the outgrowth of secondary tumors at distant sites (Kessenbrock et al., 2010). This occurrence which is called metastases, represents the end product of the invasion-metastasis cascade, and involves dissemination of cancer cells to anatomically distant organ sites and their subsequent adaptation to foreign tissue microenvironments (Valastyan and Weinberg, 2011). The most important physiological barrier to the metastasis of tumor cells is invasion of tumor cells into the extracellular matrix (ECM) and basement membrane (BM), so the degradation of ECM and BM is one of the most important steps in the process of cancer invasion and metastasis (Steeg, 2006; Wan et al., 2013).

Matrix metalloproteinases (MMPs), also known as matrixins, are a large group of zinc-dependent proteinases responsible for cleaving and rebuilding ECM and BM components such as collagen, elastin, gelatin and casein (Zitka et al., 2010). MMP-2 and MMP-9 play an important role in degradation of ECM and BM proteins because of their ability to destroy type IV collagen (Ruokolainen et al., 2004). In fact, MMP-2 and MMP-9 have already been shown to contribute to the establishment of metastasis-prone sites at tumor-distant organs (Kessenbrock et al., 2010). Thus, searching for novel chemotherapeutic agents targeting MMPs with high efficacy and specificity is an important objective to overcome clinical challenges.

Hepatocellular carcinoma (HCC) is the predominant histological type of primary liver cancer, accounting for 70–85% of total liver cancer (Di Bisceglie, 2009; Jemal et al., 2011; Tateishi and Omata, 2012). HCC is a common human cancer, being the fifth most prevalent tumor type and the third leading cause of cancer-related deaths worldwide (Shlomai et al., 2014). Metastasis of HCCs occurs in about 30–50% of patients, and depends upon HCC stages (Terada and Maruo, 2013). Metastatic HCC does not respond to the cytotoxic effects of most of the current chemotherapeutic agents (Lou et al., 2012). Thus, new anti-cancer agents with specific

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cytotoxicity and reduced side-effects are needed for the clinical management of HCC.

Solamargine is a steroidal glycoalkaloid isolated from plants of the Solanaceae family, such as *Solanum nigrum* L. (Fig. 1) Ding et al., 2012. Previous studies have shown that water and polyphenol extracts of *S. nigrum* attenuated migration and invasion behaviors of HCC cells (HepG2) and mouse melanoma cells (B16-F1), respectively (Wang et al., 2010; Yang et al., 2010). It has been shown that solamargine inhibits the growth of human tumor cell lines such as colon (HT-29 and HCT-15), prostate (LNCaP and PC-3), breast (T47D and MDA-MB-231), human hepatoma (PLC/PRF/5) and JTC-26 cell lines (Liu et al., 2004), although anti-migration and anti-invasion effects of solamargine on cancer cells remain unknown. Therefore, in this study we aimed to evaluate the inhibitory effects of solamargine on HCC cell migration and invasion in view of its molecular mechanism through determination of the expression and function of MMP-2 and MMP-9 proteins.

2. Materials and methods

2.1. Test drug

Solamargine was purchased from Glycomix Ltd. (Whiteknights, United Kingdom) and dissolved in DMSO (Dimethyl sulfoxide) and PBS to a final concentration of 870 μM and stored at −20 °C. The drugs were freshly diluted to their final concentration in culture medium prior to the start of the experiment.

2.2. Reagents

Roswell Park Memorial Institute medium (RPMI 1640) and fetal bovine serum (FBS) were purchased from Gibco Industries Inc. (Vienna, Austria). The anti-MMP-2 and anti-MMP-9 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, US). Anti-β-actin antibody, DMSO, trypan blue and triton X-100, pyruvate, penicillin G and streptomycin were obtained from Sigma Co. (Steinheim, Germany). HRP-conjugated secondary antibody was purchased from Abcam Company (Boston, US). Microcentrifuge concentrators were obtained from Millipore (Hessen, Germany). Matrigel was purchased from BD Biosciences (California, USA). 6-well invasion chambers were purchased from SPL life Science (Pocheon-si, South Korea).

2.3. Cell culture

The human hepatocellular carcinoma cell line HepG2 was cultured in RPMI 1640 supplemented with 2 mM L-glutamine, heat inactivated FBS 10% (v/v), 2 g/L sodium bicarbonate, penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37 °C in a humidified atmosphere containing 5% CO_2.

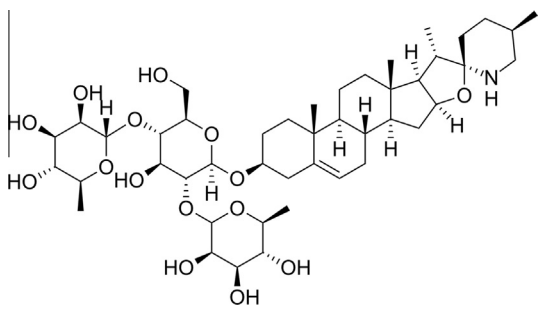

Fig. 1. Chemical structure of solamargine (Sun et al., 2010).

2.4. Cytotoxicity assay

The cytotoxic effect of solamargine was evaluated by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) colorimetric assay (Kalalinia et al., 2011). Briefly, HepG2 cells were seeded in 96-well culture plates at a density of 10^4 cells per well. After 24 h of incubation, the medium in the 96-well plate was replaced with 100 μL of new medium containing various concentrations of solamargine (0–7.5 μM) and incubated for 24 h. Control wells contained DMSO at equal volumes to those used for the test compounds. At the end of the incubation, medium was replaced with 100 μL of MTT solution (0.5 mg/ml) and the plates were incubated for 4 h at 37 °C. The purple MTT formazan crystals were dissolved in 100 μL DMSO and absorbance was determined on an ELISA plate reader (BioTek, Bad Friedrichshall, Germany) with a test wavelength of 570 nm and a reference wavelength of 630 nm to obtain the sample signal. Each experiment was repeated independently 3 times in or in triplicate (MTT assay).

2.5. Migration assay

The anti-migration effect of solamargine on HepG2 cells was measured by a wound healing migration assay in two ways: pretreatment and treatment as described by Valster et al. (2005). For the treatment wound healing migration assay, HepG2 cells (5×10^5) were grown to 90% confluence in a 6-well plate at 37 °C, 5% CO_2 incubator. The monolayers were scratched with a yellow micropipette tip (100 μM micropipette tip), washed with PBS to remove floating cell debris, and then incubated in the medium containing 0–7.5 μM of solamargine for 24 h. For the pretreatment wound healing migration assay, HepG2 cells (4×10^5) were plated in 6-well plates for 24 h. Cells were then incubated with various concentrations of solamargine (0–7.5 μM) for 24 h. Wounds were then made similar to the methods described above and cells were incubated in the medium for 24 h. Finally, after photography, wounds sizes were measured using ImageJ software, version 1.46r.

2.6. Invasion assay

The effect of solamargine on the invasiveness of HepG2 cells was determined using a modified Boyden chamber technique with matrigel-coated membranes. Briefly, 6-well transwell inserts with 8 μm polycarbonate pore size filters were coated with a uniform layer of Matrigel Basement Membrane Matrix (3 mg/ml) at 37 °C for 1 h. The HepG2 cells (4×10^5 cells/1.5 ml RPMI-1640) were then placed onto the upper compartment and incubated with solamargine (0–7.5 μM) and the lower chamber containing 10% FBS as a chemoattractant. The plates were then incubated at 37 °C for 24 h. Afterward MTT solution was added to each upper and lower well for an additional 3 h. The purple MTT formazan crystals were dissolved in 100 μL DMSO and absorbance was determined on an ELISA plate reader (BioTek, Bad Friedrichshall, Germany) with a test wavelength of 570 nm and a reference wavelength of 630 nm to obtain the sample signal. Finally, the numbers of invaded cells on the bottom of each insert were measured by MTT assays using a standard curve prepared from serial dilutions of the corresponding cell line (Mansouri et al., 2014).

2.7. Western blot analysis

The association of the expression of MMP-2 and MMP-9 with mode of tumor invasion and metastasis involvement has previously been shown in cancer cells (Kim et al., 2013). HepG2 cells (4×10^5) were plated in 6-well plates for 24 h. Then HepG2 cells were incubated with various concentrations of solamargine (0–