Ginsenoside-Rb1 as an anti-cancer therapeutic: abridged secondary publication

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KEY MESSAGES

1. Ginsenoside Rb1 and its derivative compound K target cancer stem cells.
2. Ginsenoside Rb1 and compound K chemosensitise cancer stem cells to chemotherapeutic drugs.
3. Wnt/β-catenin signalling causes the ensuing cytotoxic effects.

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Introduction

Recurrence of cancer despite chemotherapy is associated with high mortality. Cancer stem cells (CSCs) have been reported to possess properties of self-renewal, differentiation, and drug resistance that lead to tumorigenesis and chemoresistance.[1] They are not eliminated by conventional chemotherapy owing to their distinct molecular signatures. Thus, understanding CSCs' biology and targeting them are important.

Saponins have been shown to exhibit potent cytotoxic effects as chemotherapeutics.[2] Ginsenoside-Rb1 isolated from ginseng (0.37%-0.5%) is a notable saponin. After being taken orally, about 70% of Rb1 is metabolised to 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol (compound K) by gut microbes.[3] This metabolite can be readily absorbed into the blood and retained in the body.

Methods

SKOV-3 and HEYA8 CSCs or primary CSCs derived from patients with ovarian carcinoma were isolated in serum-free stem cell-selective conditions and cultured as previously described.[4] To assess tumour recurrence, primary spheres were dissociated as single cells and replated for sphere formation by secondary passaging.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to analyse the rates of proliferation/survival based on the manufacturer's protocols (Sigma, St Louis [MO], US). The quantity of formazan was measured at a wavelength of 570 nm using a microplate reader (Bio-Rad, Hercules [CA], US).

Equal amounts of proteins were separated by SDS-PAGE, transferred onto nitrocellulose membrane, and blocked with 5% non-fat milk for 1 hour at room temperature. Membranes were then incubated with the primary antibody overnight at 4°C, followed by incubation with horseradish peroxidise-conjugated secondary antibodies for 3 hours at room temperature (Bio-Rad, Hercules [CA], US). β-actin was included as a loading control. The target proteins were detected by Amersham enhanced chemiluminescent detection reagents (GE Healthcare, Little Chalfont, UK).

All animal experiments were approved by the University of Hong Kong Institutional Animal Care and Use Committee. Cells (10^6 cells) were inoculated subcutaneously into the right flank of BALB/c athymic nude mice. When tumour size reached approximately 50 mm^3, the mice were randomly divided into nine groups and administered DMSO, Rb1, compound K, cisplatin, paclitaxel, Rb1 + cisplatin, Rb1 + paclitaxel, compound K + cisplatin, or compound K + paclitaxel by oral gavage. Tumour volume and body weight were measured twice weekly for 28 days. Blood samples were collected from the retro-orbital sinus to test enzyme levels indicative of cardiac, hepatic, and renal function. Organs were paraffin embedded and stained with haematoxylin and eosin for histologic examination.

One-way analyses of variance were used to compare different treatment groups. The Tukey test was performed to detect differences. P values of <0.05 were considered statistically significant.

Results

MTT assay was used to examine the cytotoxic effects of Rb1 and its metabolite compound K on SKOV-3 and HEYA8 CSCs. Rb1 and compound K inhibited tumour sphere formation and growth of both SKOV-3 and HEYA8 CSCs in a dose-dependent manner. The lethal concentrations that led to 50% survival in SKOV-3 cells were 0.25 μM for Rb1 and 0.1 μM for compound K, whereas in HEYA8 cells, the corresponding lethal concentrations were 0.23 μM for Rb1 and 0.125 μM for compound K. In addition, 250 nM Rb1 and 125 nM compound K suppressed tumour sphere formation and growth of SKOV-3 and HEYA8 CSCs in a time-dependent manner (measured at 0, 24, and 48 hours). Moreover, Rb1 and compound K blocked the regrowth of

secondary spheres. The expression of CSC markers such as Bmil-1, Oct4, and Nanog were reduced by Rb1 or compound K, with maximal effects after 48 hours. These results suggest that Rb1 and compound K inhibit CSC self-renewal.

We further investigated the effects of Rb1 and compound K on chemotherapeutics. In SKOV-3 and HEYA8 CSCs, Rb1 or compound K sensitised CSCs to clinically relevant doses of cisplatin (50 μM) and paclitaxel (100 nM).[5] Consistently, the expression levels of Bmi-1, Oct4, and Nanog decreased. In contrast, SKOV-3 and HEYA8 CSCs were resistant to both cisplatin and paclitaxel without treatment by Rb1 or compound K.

To explore the underlying mechanism by which Rb1 and compound K regulate CSC chemosensitisation, the key role of Wnt/β-catenin signalling in CSC self-renewal and carcinogenesis was determined. Rb1 and compound K significantly inhibited β-catenin expression concomitant with a decrease in β-catenin/TCF transcriptional activity via TOPFLASH activation. There was a decrease in the expression of two β-catenin/TCF targets and pivotal drug transporters ABCG2 and P-glycoprotein. Nonetheless, expression of a stable mutant form of β-catenin (S37A) reverted the chemosensitising effect of Rb1 and compound K in CSCs. Similar results were obtained by expressing a constitutively active construct of TCF (VP16-TCF). These results suggest that Rb1 and compound K target the Wnt/β-catenin-ABCG2 and P-glycoprotein signalling pathway.

An in vivo study was performed to further assess the effects of Rb1 and compound K. Tumour growth was significantly inhibited by Rb1 or compound K compared with vehicle controls. The combined treatment of Rb1 or compound K with cisplatin or paclitaxel led to a marked decrease in tumour size. There was no noted weight loss in the animals, and there was no histological or enzymatic alteration in the heart, kidney, liver, lungs, and spleen compared with controls. These results suggest that Rb1 and compound K could be used as anti-CSC agents with no obvious adverse effects.

Discussion

Chemoresistance is a major factor that leads to failure of anti-cancer therapies. In this study, Rb1 and compound K significantly inhibited the growth of CSCs and enhanced sensitivities to cisplatin and paclitaxel through the Wnt/β-catenin-ABCG2/P-glycoprotein signalling pathway. To our knowledge, this is the first evidence that ginseng affects CSC chemosensitisation. Combined therapy could be beneficial for refractory/recurrent tumours.

Rb1 and compound K could be promising therapeutic and chemopreventive agents. They exhibit better bioavailability than most other saponins, and they show various anti-carcinogenic activities such as growth inhibition, apoptosis, and anti-angiogenesis. They inhibit many tumour-promoting activities and seem to improve normal cellular functions.

Cisplatin and paclitaxel are major anti-cancer drugs for treatment of ovarian cancer. Nonetheless, drug resistance remains a major challenge. Rb1 and compound K could sensitise CSCs, the drug-resistant subpopulation of cancer cells, to clinical doses of cisplatin and paclitaxel chemotherapeutic treatments.

Ginseng has been considered to have low toxicity, and overdose is well tolerated. Rb1 and compound K should be further investigated as an anti-cancer therapeutics, as there was no organ toxicity *in vivo*.

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Disclosure

The results of this research have been previously published in:

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