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Research article

Ginsenoside Rh2 inhibiting HCT116 colon cancer cell proliferation through blocking PDZ-binding kinase/T-LAK cell-originated protein kinase

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A B S T R A C T

Background: Ginsenoside Rh2 (GRh2) is the main bioactive component in American ginseng, a commonly used herb, and its antitumor activity had been studied in previous studies. PDZ-binding kinase/T-LAK cell-originated protein kinase (PBK/TOPK), a serine/threonine protein kinase, is highly expressed in HCT116 colorectal cancer cells.
Methods: We examined the effect of GRh2 on HCT116 cells *ex vivo*. Next, we performed *in vitro* binding assay and *in vitro* kinase assay to search for the target of GRh2. Furthermore, we elucidated the underlying molecular mechanisms for the antitumor effect of GRh2 *ex vivo* and *in vivo*.
Results: The results of our *in vitro* studies indicated that GRh2 can directly bind with PBK/TOPK and GRh2 also can directly inhibit PBK/TOPK activity. *Ex vivo* studies showed that GRh2 significantly induced cell death in HCT116 colorectal cancer cells. Further mechanistic study demonstrated that these compounds inhibited the phosphorylation levels of the extracellular regulated protein kinases 1/2 (ERK1/2) and (H3) in HCT116 colorectal cancer cells. *In vivo* studies showed GRh2 inhibited the growth of xenograft tumors of HCT116 cells and inhibited the phosphorylation levels of the extracellular regulated protein kinases 1/2 and histone H3.
Conclusion: The results indicate that GRh2 exerts promising antitumor effect that is specific to human HCT116 colorectal cancer cells through inhibiting the activity of PBK/TOPK.
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1. Introduction

PDZ-binding kinase/T-LAK cell-originated protein kinase (PBK/TOPK), a serine–threonine mitogen-activated protein kinase, is a member of the MEK protein family [1,2]. It is involved in the mitotic checkpoint of cells [3], DNA damage [4], tumor transformation and metastasis [5,6], and inflammation [7]. Previous studies showed that PBK/TOPK is highly expressed in many cancers such as lymphoma, leukemia, melanoma, breast cancer, lung cancer, cholangiocarcinoma, glioma, and colorectal cancer [8–14]. It may play an important role in prognostic and predictive diagnosis and therapy in cancer. In addition, it was reported that PBK/TOPK is highly

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expressed in HCT116 colorectal cancer cells [14,15], and the positive feedback loop between PBK/TOPK and extracellular regulated protein kinase (ERK) 2 increases tumorigenesis of HCT116 colorectal cancer cells [14]. Also, studies have shown that PBK/TOPK could phosphorylate histone H3 (H3) at Ser10 *in vitro* and *in vivo* [12]. PBK/TOPK may be a valid target for antineoplastic kinase inhibitors to sensitize tumor cells to chemotherapy-induced apoptosis and growth suppression [15]. However, there are few PBK/TOPK inhibitors reported in basic research. One PBK/TOPK inhibitor HI-TOPK-032 [16], OTS964 [17], has great suppression in colon cancer and other cancers, but has significant side-effects [17]. Therefore, we aimed to look for a traditional medicine to inhibit PBK/TOPK activity.

Ginsenosides are the main active chemical constituents of ginseng, the root of *Panax ginseng* Meyer [18], which has great pharmacological efficacy to promote the human health in global traditional medicine, such as antidiabetic, anti-inflammatory, antitumor, anti-UV radial, protection of nerves, regulation of blood pressure, and other functions [19–22]. Ginsenosides could be classified in three types based on aglycone moieties: protopanaxadiol-type ginsenosides, protopanaxatriol-type ginsenosides, and oleanolic acid-type ginsenosides [22]. Ginsenoside Rh2 (GRh2), identified as an protopanaxadiol-type ginsenoside, exhibits significant antitumor effects on pancreatic cancer, leukemia, prostatic cancer, skin squamous cell carcinoma, glioblastoma, and so on [23–27]. Some protopanaxadiol-type ginsenosides, such as ginsenosides Rb1/2 and Rg3, are metabolized to GRh2 by human intestinal bacteria [28]. GRh2 exists in two stereoisomeric forms, 20(R)-GRh2 and 20(S)-GRh2 (Fig. 1A). Compared to 20(R)-GRh2, 20(S)-GRh2 displays an evident anticancer activity through suppressing cell proliferation in human lung adenocarcinoma A549 cells [29], and inhibiting proliferation of androgen-dependent or independent prostate cancer cells [30].

Significant antitumor effects of ginsenosides are observed in colorectal cancer cells [31,32]. However, its detailed mechanism remains unclear. The present study is the first to investigate whether GRh2 effectively targets PBK/TOPK by directly binding to PBK/TOPK, resulting in the inhibition of HCT116 cells *in vivo* and *in vitro*.

2. Materials and methods

2.1. Reagents and antibodies

HCT116 colorectal cancer cell lines were purchased from ATCC (Manassas, VA, USA). Commercial GRh2 was obtained from Weikeqi Bioscience (Chengdu, China). The GRh2 was prepared in a stock of 100 mg/mL and applied to cultured HCT116 cells at 5μM, 15μM, 25μM, and 50μM. PBK/TOPK small hairpin RNA sequence is 5′-CCGGGGGAACTAGGCCACCTATTAACTCGAGTTAATAGGTGGCCTAGT-TCCCTTTTTG-3′ and 5′-AATTCAAAAAGGGAACTAGGCCACCTAT-TAACTCGAGTTAATAGGTGGCCTAGTTCCC-3′. Active PBK/TOPK, active ERK2, inactive ERK2, H3 protein for kinase assays were purchased from Millipore (Billerica, MA, USA). Antibodies to detect total PBK/TOPK, phospho-PBK/TOPK (p-PBK/TOPK; T9), total ERK1/2, phospho-ERK1/2 (p-ERK1/2; T202/Y204), total H3 and phospho-H3 (p-H3; S10) were purchased from Cell Signaling Technology (Danvers, MA, USA). Antibodies to detect β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Cell culture and MTS assay

HCT116 colorectal cancer cell lines were cultured at 37°C in a 5% CO_2 incubator in McCoy' s 5A medium containing 10% fetal bovine serum (FBS). The cells were starved for 24 h before the addition of 20 ng/mL epidermal growth factor (EGF) in McCoy's 5A medium without serum.

To estimate cytotoxicity, cells were seeded (8×10^3 cells per well) in 96-well plates and cultured overnight. Cells were then fed with fresh medium and treated with different concentrations of

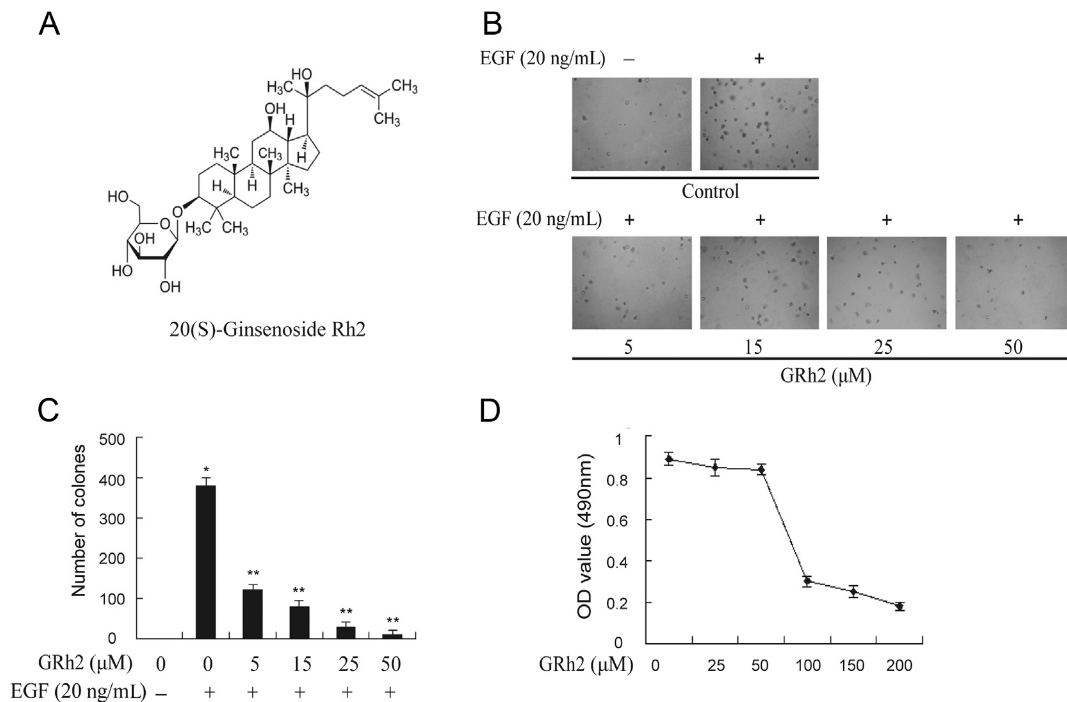

Fig. 1. Chemical structure of GRh2 and its effect on EGF-induced neoplastic transformation and signal transduction in HCT116 cells. (A) Chemical structure of GRh2. (B) GRh2 inhibits EGF-induced anchorage-independent growth of HCT116 cells in a dose dependent manner. (C) Histogram shown as means ± SD of values from three independent experiments. * Significant compared with control alone, $p < 0.05$. ** Significant compared with EGF alone, $p < 0.05$. (D) Cytotoxic effects of GRh2 on HCT116 cells. An MTS assay was used after different concentration of GRh2 treatment for 24 h.

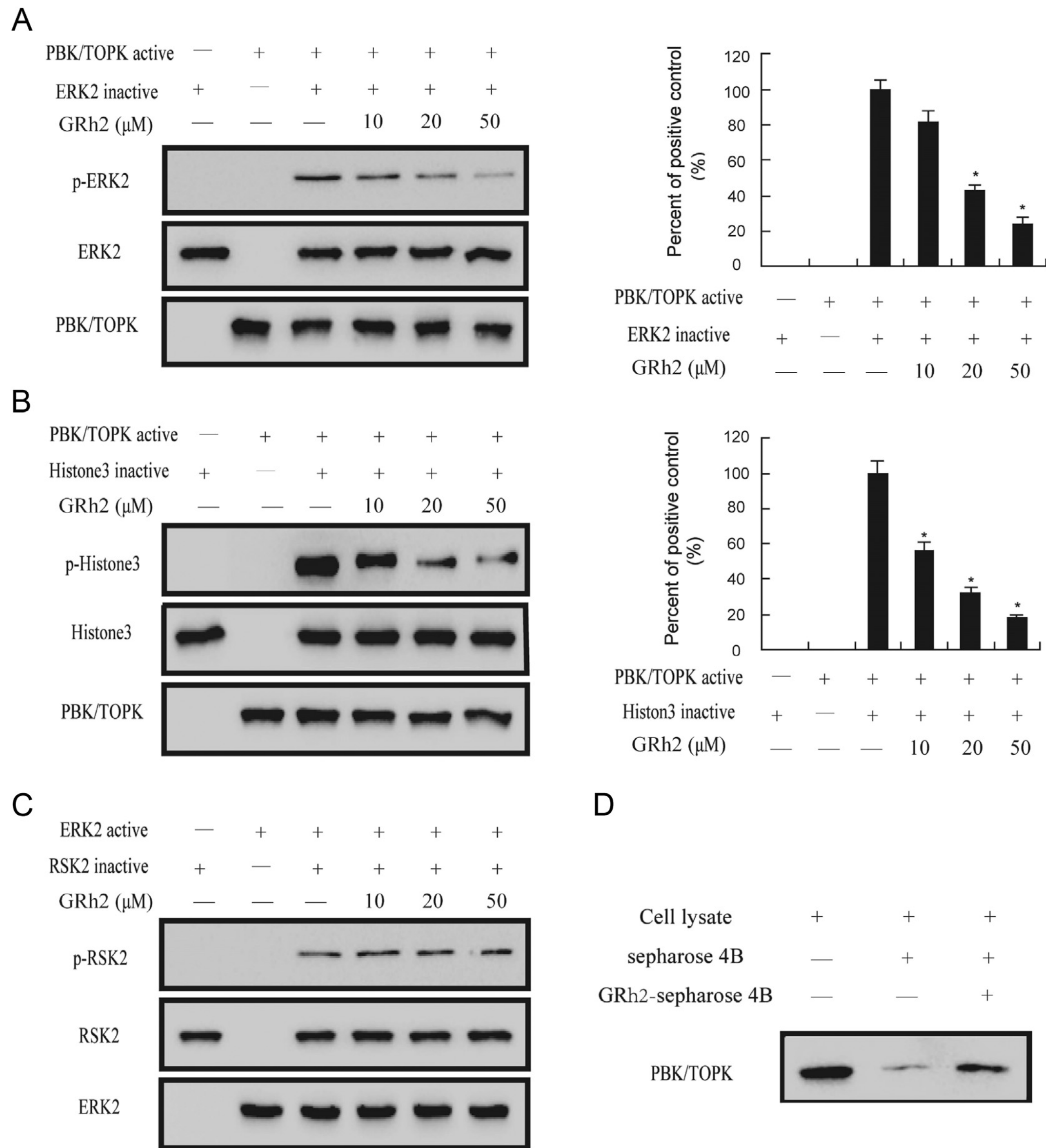

Fig. 2. GRh2 inhibits TOPK activity by directly binding with TOPK. (A, B) GRh2 inhibits TOPK activity *in vitro* kinase assays in a dose dependent manner. Inactive (A) ERK2 and (B) histone H3 were used as the substrate for *in vitro* kinase assays with active TOPK and 100μM ATP. Proteins were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and detected by Western blot using specific phosphor-antibody. The asterisk indicates a significant difference between groups treated with different concentration of GRh2 and the DMSO alone (* $p < 0.05$). (C) GRh2 has no effects on the ERK2 activity. (D) Sepharose 4B was used for binding and pull-down assays as described in the Materials and methods section.

GRh2. After culturing for various times, the cytotoxicity of GRh2 was measured using an MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2H-tetrazdium] assay kit (Promega, Madison, WI, USA) according to the manufacturer's instructions.

2.3. Western blot

HCT116 colorectal cancer cells (1.5×10^6) were cultured for 48 h and starved in serum-free medium for 24 h without FBS. The cells were treated with GRh2 (5μM, 15μM, 25μM, 50μM) for 3 h, 6 h, and 12 h before exposure to 20 ng/mL EGF. Harvested cells were disrupted and protein concentrations were determined by the Bradford method. Lysate protein was subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride membranes, which were incubated with a specific primary antibody at 4°C for overnight. Proteins were visualized using a chemiluminescence detection kit after hybridization with a horseradish peroxidase-conjugated secondary antibody.

2.4. Anchorage-independent transformation assay

The cells (8×10^3/mL) were exposed to EGF (20 ng/mL) and treated with GRh2 (0–50μM) in 1 mL of 0.3% Basal Medium Eagle agar containing 10% FBS, 2mM L-glutamine, and 25 μg/mL gentamicin. The cultures were maintained at 37°C, in a 5% CO_2 incubator for 7–14 d, and the colonies were counted under a microscope with the aid of Image-Pro Plus v.4 (Media Cybernetics, Silver Spring, MD, USA).

2.5. Immunohistochemistry

For p-ERK and p-H3 immunostaining, antigen retrieval was performed by microwave after deparaffinization and rehydration of

tissue sections (5 μm) for 10 min in sodium citrate buffer. The sections were cooled to room temperature, treated with 3% H_2O_2 in methanol for 10 min and blocked with 6% horse serum for 40 min at room temperature. The sections were incubated at 4°C overnight with primary antibodies. The sections were washed in phosphate-buffered saline and incubated with the secondary antibody for 30 min. After washing, color was developed by the indirect avidin/biotin-enhanced horseradish peroxidase method with the horseradish peroxidase kit and with 3,3-diaminobenzidine tetrahydrochloride as substrate. For evaluation, photomicrographs were taken with a digital camera. The positively stained cells within each photomicrograph were counted.

2.6. In vitro kinase assay

Inactive ERK2 and H3 proteins were used as the substrate for an *in vitro* kinase assay with active PBK/TOPK. Firstly, active PBK/TOPK was incubated with GRh2 (10μM, 20μM, and 50μM) in 1×kinase buffer (25mM Tris-HCl pH 7.5, 5mM β-glycerophosphate, 2mM dithiothreitol, 0.1mM Na_3VO_4, 10mM $MgCl_2$) at 30°C for 10 min. Then, inactive substrate and 100μM ATP were added to reaction and incubated at 32°C for 1 h. Reactions were stopped and proteins detected by Western blotting.

2.7. In vitro binding assay

HCT116 cell lysates (1 mg) were incubated with the GRh2, or GRh2-Sepharose 4B beads in the reaction buffer [50mM Tris (pH 7.5), 5mM ethylenediaminetetraacetic acid, 150mM NaCl, 1mM dithiothreitol, 0.01% Nonidet P-40, 2 μg/mL bovine serum albumin, 0.02mM phenylmethylsulfonyl fluoride, and 1 μg/mL protease inhibitor mixture]. After gentle rocking overnight at 4°C, the beads were washed five times and proteins were analyzed by Western blot.

2.8. Xenograft mouse model

Athymic nude mice (NIH Swiss nude, age 6–9 wk) were obtained from the animal center of the Fourth Military Medical University (No. 169, Changle West Road, Xi'an, Shaanxi, 710032, PR China). Mice were divided into different groups ($n = 10$ of each group). HCT116 colorectal cancer cells (4×10^6/0.1 mL) were injected subcutaneously into the right flank of each mouse. GRh2 or

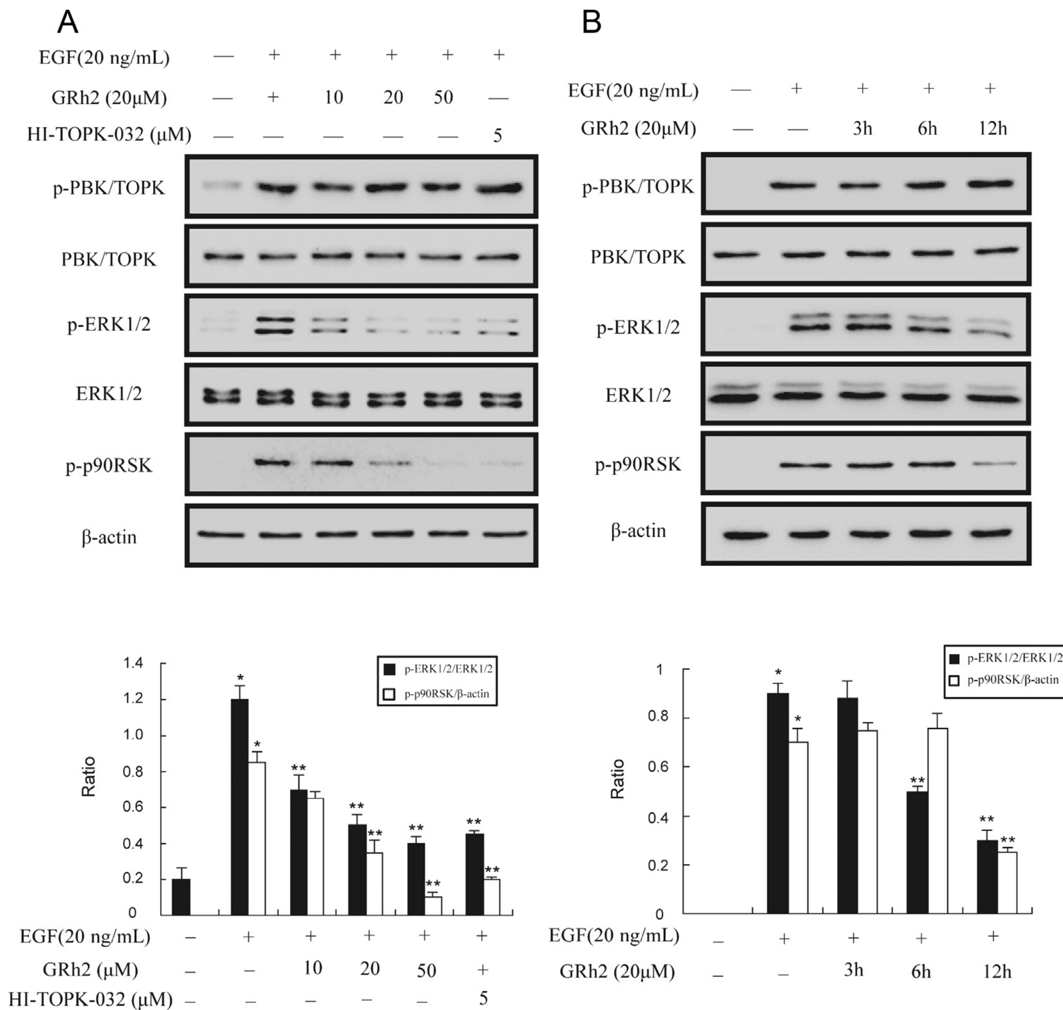

Fig. 3. TOPK is required for GRh2-inhibition of the phosphorylation of ERK1/2 and p90RSK induced by EGF in HCT116 cells in a dose- and time-dependent manner. (A) GRh2 inhibits TOPK/ERK signaling pathway in a dose-dependent manner. HCT116 cells starved in serum-free medium for 24 h, and treated with GRh2 at the indicated concentration for 12 h. After stimulation with EGF (20 ng/mL) for 15 min, cell were harvested and protein levels were determined by western blot analysis. (B) GRh2 inhibits TOPK/ERK signaling pathway in a time-dependent manner. HCT116 cells were starved in serum-free medium for 24 h, and treated with GRh2 at the indicated time for 20μM. After stimulation with EGF (20 ng/mL) for 15 min, cells were harvested and protein levels were determined by western blot analysis using specific antibodies. The phosphorylation level of ERK1/2 and p90RSK was quantified by scanning densitometry and normalized to ERK1/2 or β-actin protein. The values shown are mean ± standard error of the mean of data from three independent experiments. * Significant compared with control alone, $p < 0.05$.** Significant compared with EGF alone, $p < 0.05$.

vehicle was injected three times a week for 29 d. Tumor volumes and body weights were measured. Tumor tissues from mice were embedded in a paraffin block and subjected to immunohistochemistry or hematoxylin and eosin staining.

2.9. Statistical analysis

All quantitative data are expressed as mean values ± standard deviation, and significant differences were determined by Student *t* test or by one-way analysis of variance in SPSS version 13.0 software (SPSS Inc., Chicago, IL, USA). A probability value of $p < 0.05$ was used as the criterion for statistical significance.

3. Results

3.1. GRh2 suppresses HCT116 cells induced by EGF to anchor the dependency proliferation

In the present study, we first examined the effect of GRh2 on EGF-induced neoplastic transformation of HCT116 cells. Treatment of HCT116 cells with GRh2 significantly reduced EGF-induced neoplastic transformation in soft agar in a dose-dependent manner (Fig. 1B). GRh2 at 25μM or 50μM caused a reduction to 62% or 85% of control, respectively, and the difference was statistically significant ($p < 0.05$; Fig. 1C). We can see that the viability of HCT116 cells was not affected by the 50μM GRh2, while many dead HCT116 cells were seen when GRh2 concentration was 100μM (Fig. 1D). The results show that GRh2 significantly inhibits HCT116 cells growth in soft agar in a dose-dependent manner in the effective concentration range of GRh2.

3.2. GRh2 is a potent inhibitor of PBK/TOPK activity and binds directly with PBK/TOPK

To identify a potent inhibitor of PBK/TOPK, we examined more than 12 compounds that have antitumor effect using the *in vitro* kinase assay. We identified GRh2 as a good inhibitor of PBK/TOPK. To determine the effects of GRh2 on PBK/TOPK activity we used ERK2 as the substrate of PBK/TOPK. The phosphorylation of EKR2 was strongly inhibited by GRh2 in a dose-dependent manner. For example, 20μM GR2h resulted in about 58% inhibition of PBK/TOPK activity and 50μM caused about 78% inhibition of PBK/TOPK activity (Fig. 2A). We also used inactive H3 as a substrate for PBK/TOPK and results indicated that the phosphorylation of H3 on Ser10 was also dose dependently suppressed by GRh2 treatment. GRh2 at 20μM resulted in about 63% inhibition of PBK/TOPK activity and 50μM caused about 80%; the difference was statistically significant ($p < 0.05$; Fig. 2B). In addition, we investigated whether GRh2 inhibited the ERK2 activity; the result clearly shows that GRh2 does not directly inhibit ERK2 activity (Fig. 2C). These results notably support that GRh2 directly inhibits PBK/TOPK activity.

The *in vitro* kinase assay results show that GRh2 inhibits PBK/TOPK activity, which implies direct binding between GRh2 and PBK/TOPK. To confirm this idea, we performed an *in vitro* binding assay using GRh2-conjugated sepharose 4B beads or sepharose 4B beads alone in HCT116 cells lysate that had been verified to express PBK/TOPK highly. A strong band representing PBK/TOPK was observed in the GRh2-conjugated beads group, whereas no obvious band was seen in the beads alone group (Fig. 2D). The results clearly indicate that GRh2 could directly bind with PBK/TOPK protein. Therefore, these above results show that GRh2 could directly bind to PBK/TOPK and inhibit PBK/TOPK activity.

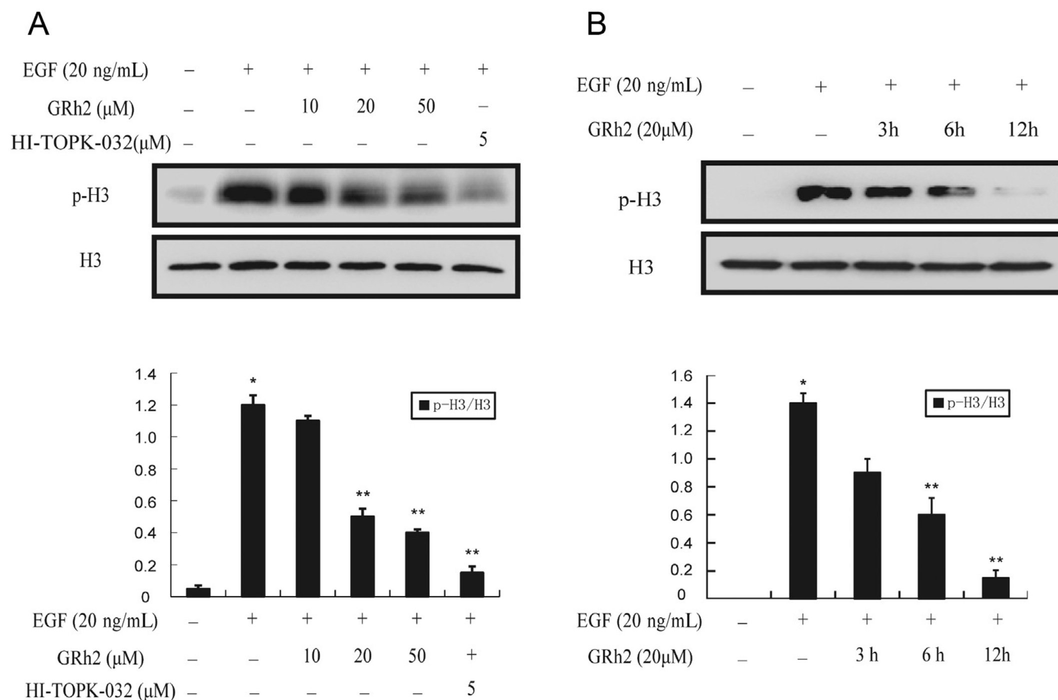

Fig. 4. TOPK is required for GRh2 inhibition of the phosphorylation of histone H3 induced by EGF in HCT116 cells in a time- and dose-dependent manner. (A) GRh2 inhibits TOPK/H3 signaling pathway in a dose-dependent manner. HCT116 cells were starved in serum-free medium for 24 h, and treated with GRh2 at the indicated concentration for 12 h. After stimulation with EGF (20 ng/mL) for 15 min, cells were harvested using the extraction method of histones, and protein levels were determined by western blot analysis. (B) GRh2 inhibits TOPK/H3 signaling pathway in a time-dependent manner. HCT116 cells were starved in serum-free medium for 24 h, and treated with GRh2 at the indicated time for 20μM. After stimulation with EGF (20 ng/mL) for 15 min, cell were harvested using the extraction method of histones, and protein levels were determined by western blot analysis. The phosphorylation level H3 was quantified by scanning densitometry and normalized to total H3 protein. The values shown are mean ± standard error of the mean of data from three independent experiments. * Significant compared with control alone, $p < 0.05$. ** Significant compared with EGF alone, $p < 0.05$.

3.3. GRh2 inhibits the phosphorylation of ERK1/2 in a dose-dependent manner in HCT116 cells

Previous studies suggested that PBK/TOPK/ERK2 signaling pathway is highly activated in human colorectal cancer [14]. Therefore, we examined p-PBK/TOPK, p-ERK1/2, and p-p90RSK, which is the downstream signaling pathway of ERK1/2 in HCT116 cells. HI-TOPK-032, a well-known inhibitor of PBK/TOPK, was used as a positive control in the experiment [16]. Western blot results suggested that phosphorylation of PBK/TOPK has no significant change. However, the phosphorylation of ERK1/2 in HCT116 cells was strongly suppressed by GRh2 in a dose- and time-dependent manner at the condition of EGF (20 ng/mL) stimulation. For example, 10μM GR2h caused about 43% inhibition of the phosphorylation of ERK1/2, and 20μM and 50μM GR2h resulted in about 67% and 81% inhibition, respectively, of the phosphorylation of ERK1/2 after 12 h (Fig. 3). Phosphorylation of p90RSK was also strongly suppressed by GRh2. For example, 20μM GR2h induced about 85% inhibition of p90RSK phosphorylation, similar to HI-TOPK-032 (Figs. 3A and 3B). These data indicate that GRh2 suppresses the phosphorylation of ERK1/2 through inhibiting the PBK/TOPK activity in HCT116 cells.

3.4. GRh2 inhibits phosphorylation of H3 in a time- and dose-dependent manner in HCT116 cells

Previous studies confirmed that cell cycle dependent phosphorylation of H3 at Ser10 was well correlated with PBK/TOPK expression level, particularly in the early stage of mitosis. It was reported that PBK/TOPK/H3 signaling pathway may promote mitotic events, thus enhancing cancer cell proliferation [33], and endogenous PBK/TOPK phosphorylated H3 at the Ser10 site in breast cancer cells [12]. We also verified that PBK/TOPK phosphorylated H3 *in vitro*; we further detected the change of phosphorylation of H3 in HCT116 cells. HI-TOPK-032, also was used as a positive control. Western blot results showed that the phosphorylation of H3 was strongly inhibited by GRh2 in a dose- and time-dependent manner at the condition of EGF (20 ng/mL) stimulation (Fig. 4). The PBK/TOPK inhibitor HI-TOPK-032 also suppressed the phosphorylation of H3, similar to GRh2. These results show that GRh2 inhibits the phosphorylation of H3 in a dose- and time-dependent manner through inhibiting the PBK/TOPK activity in HCT116 cells.

3.5. Knockdown of PBK/TOPK in HCT116 cells decreases the sensitivity of GRh2

We then examined whether knockdown PBK/TOPK expression influences the sensitivity of HCT116 cells to GRh2. First, we determined the efficiency of PBK/TOPK knockdown by Western blot and anchorage-independent growth. The expression of PBK/TOPK was obviously decreased after knocking down PBK/TOPK compared with the control group (Fig. 5A). In soft agar the growth of HCT116 cells in the knockdown PBK/TOPK group also decreased $>$ 60% compared with the control group (Figs. 5B and 5C). HCT116 cells transfected with the shPBK/TOPK or shMock group were treated with GRh2 or control and subjected to soft agar assay. The results show that 20μM GRh2 inhibits the clone of HCT116 cells transfected with shMock by 60%. By contrast, inhibition by clone was only

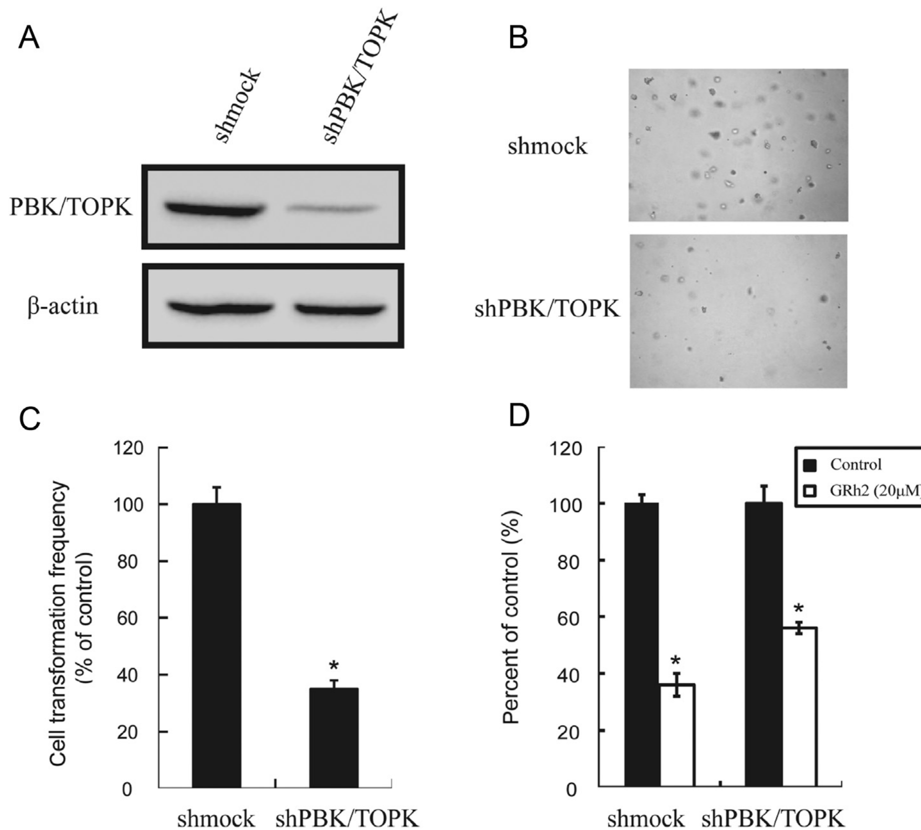

Fig. 5. Knockdown of TOPK in HCT116 cells decreases sensitivity to GRh2. (A) Efficiency of TOPK small hairpin RNA in HCT116 cells. (B) Anchorage-independent growth of HCT116 cells transfected with shMock or shTOPK. (C) The relative clone number from A was quantified by scanning densitometry and normalized to shMock. The values shown are mean ± standard error of the mean of data from three independent experiments. * Significant compared with shMock group, $p < 0.05$. (D) Sensitivity of HCT116 cells transfected with shMock or shTOPK to treatment with GRh2.

about 29% in HCT116 cells transfected with shPBK/TOPK, making clear that HCT116 cells transfected with shPBK/TOPK are more resistant to GRh2 treatment (Fig. 5D). These results indicate that PBK/TOPK plays an important role in the sensitivity of HCT116 colorectal cancer cells to the antiproliferative effects of GRh2.

3.6. GRh2 inhibits the growth of tumor in vivo

To explore the antitumor efficacy of GRh2 in xenograft model, we injected subcutaneously into the left flank of 6-wk-old athymic nude mice using HCT116 cells. Mice were then administered vehicle or GRh2 by injection of 10 mg/kg or 50 mg/kg three times a week for 26 d. The data indicate 10 mg/kg or 50 mg/kg of GRh2 significantly suppressed HCT116 tumor growth by 49% and 78% relative to the vehicle groups (Fig. 6A). However, the weight of mice had no significant difference between the vehicle and GRh2-treated group (Fig. 6B). The effect of GRh2 on PBK/TOPK protein targets was evaluated by immunochemistry and hematoxylin–eosin staining of HCT116 tumor tissues. The expression of p-ERK1/2 and p-H3 was markedly decreased after treatment with GRh2 at either 10 mg/kg or 50 mg/kg (Figs. 6C and 6D). These data further indicate that GRh2 could inhibit HCT116 tumor growth and PBK/TOPK activity *in vivo*.

4. Discussion

Previous reports showed that the serine–threonine kinase PBK/TOPK contributes to oncogenic functions including cancer development, proliferation, metastasis, and antiapoptotic effects. However, PBK/TOPK crystal structure has not been determined, which may be the constraint to PBK/TOPK inhibitor development. On the basis of preliminary anticarcinogen traditional medicine reports, we selected more than 10 traditional compounds by *in vitro* kinase assay and identified GRh2 as a potent PBK/TOPK inhibitor.

While American ginseng has been reported to have potential chemopreventative effects relevant to colon cancer [34–37], the concrete mechanism explaining its effective antitumor ingredient GRh2 has not yet been elucidated in colorectal cancer. Previous studies revealed that ginsenosides induce apoptosis and antiproliferative mechanism in HT-29 colon cancer cells, and are involved in mitotic inhibition, DNA replication, and growth factor signaling pathway [38,39]. Our study also showed that GRh2 can promote HCT116 cells apoptosis in the optimal concentration of GRh2. The anchorage-independent cell transformation assay revealed that GRh2 effectively inhibits EGF-induced transformation of HCT116 cells in a dose-dependent manner. In addition, another report showed that HCT116 cells treated with American ginseng were arrested in the G0/G1 phase of the cell cycle and the expression of p53 and p21 proteins were increased, while p-MEK was decreased [40]. PBK/TOPK, like constitutively activated MEK, is overexpressed in many malignant cancer cells, especially HCT116 colorectal cancer cells [14,15]; there is a positive feedback loop between PBK/TOPK and ERK2 that increases tumorigenesis properties of HCT116 colorectal cancer cells [14]. PBK/TOPK can also phosphorylate H3 at Ser10 site to promote mitotic events, and then

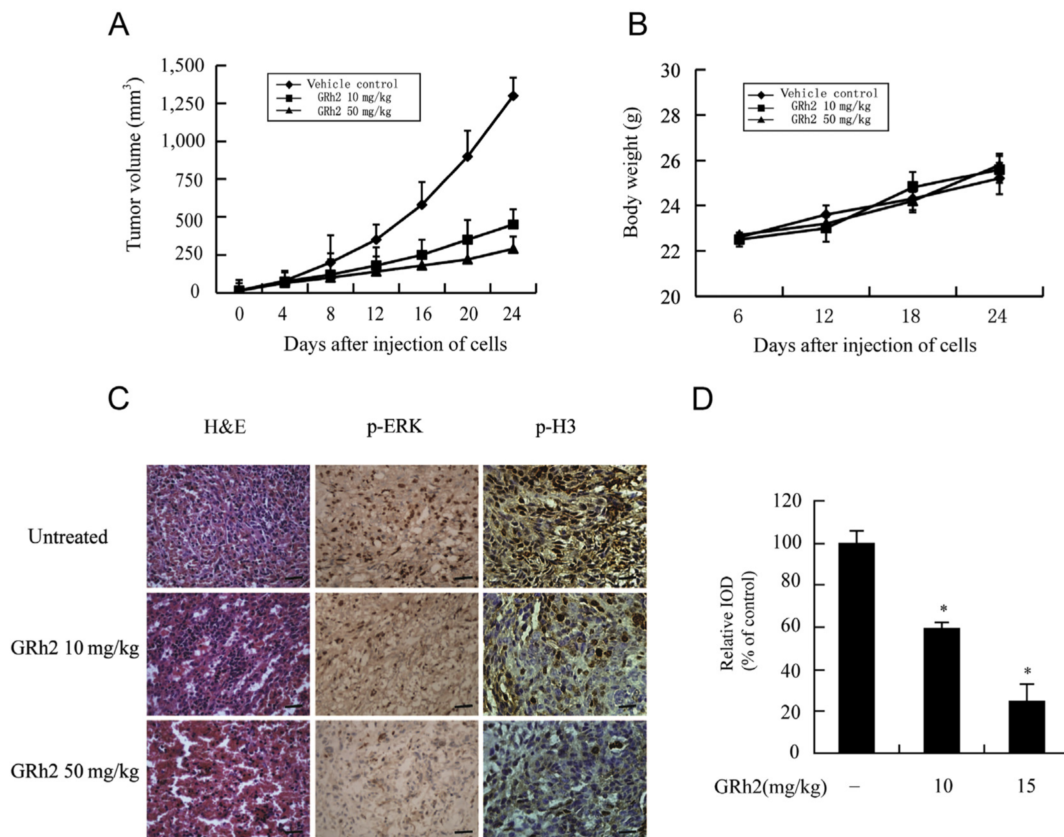

Fig. 6. Efficiency of GRh2 on colon cancer growth and TOPK targets in a HCT116 xenograft mouse model. (A) GRh2 significantly suppresses colorectal cancer cell growth. The average volume of mice were plotted over 24 d after tumor cell injection. Data are shown as mean ± standard error of the mean from three independent experiments. * Significant compared with control, $p < 0.05$. (B) GRh2 has no effect on mouse body weight. Body weights of mice were measured once every 6 d. (C) Hematoxylin and eosin staining and immunohistochemistry analysis of tumor tissues. Tumor tissue slides were prepared with paraffin sections after fixation with formalin and then stained with hematoxylin and eosin, p-ERK antibody, and p-H3 antibody. (D) Quantification of expression of p-ERK and phospho-H3 in tumor tissues using the Image-Pro Plus software program and data are shown as integrated optical density units.

enhancing cancer cell proliferation and transformation [12]. PBK/TOPK reportedly plays an important role in the negative regulation of P53 expression in HCT116 colorectal cancer cells [15]. GRh2 induced apoptosis and paraptosis-like cell death in colorectal cancer HCT116 cells through activation of p53 [41]. Thus, the PBK/TOPK signaling pathway is likely to play an important role in the antitumor activity of GRh2.

We determined whether GRh2 could inhibit the PBK/TOPK activity using ERK2 and H3 as a substrate of PBK/TOPK in an *in vitro* kinase assay. The results showed that GRh2 inhibited the PBK/TOPK activity in a dose dependent manner, while the ERK2 activity was not influenced by GRh2. We further investigated whether GRh2 mediated the PBK/TOPK signaling pathway in HCT116 cells; the results clearly showed that GRh2 could reduce the expression level of p-ERK1/2 and p-H3 in a dose-dependent manner. In addition, knocking down PBK/TOPK expression reduced the sensitivity of HCT116 cells to GRh2 treatment. Furthermore, *in vivo* studies of xenograft mouse model indicate that GRh2 inhibited PBK/TOPK activity in tumor tissues resulting in inhibition of tumor growth.

In conclusion, the present study identified that PBK/TOPK is a direct and important target of GRh2 for suppression of colon cancer proliferation and transformation. These studies also suggest that American ginseng might have potential value in colorectal cancer chemoprevention, and provide a better understanding of the mechanisms for the beneficial effects of American ginseng in human health.

Conflicts of interest

The authors have declared that there is no conflict of interest.

Acknowledgments

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