Research Article

20 (S)-ginsenoside Rh2 inhibits colorectal cancer cell growth by suppressing the Axl signaling pathway in vitro and in vivo

Haibo Zhang a,1, Jun-Koo Yi b,1, Hai Huang a, Sijun Park c, Wookbong Kwon d, Eunghyang Kim a, Soyoung Jang f, Si-Yong Kim c, Seong-kyoon Choi d, e, Duhak Yoon a, Sung-Hyun Kim f, Kangdong Liu g, Zigang Dong g, Zae Young Ryoo c, Myoung Ok Kim a, *  

a Department of Animal Science and Biotechnology, ITRD, Kyungpook National University, Sangju, Republic of Korea  
b Gyeongbuk Livestock Research Institute, Yeongju, Republic of Korea  
c School of Life Sciences, BK21 FOUR KNU Creative BioResearch, Kyungpook National University, Daegu, Republic of Korea  
d Division of Biotechnology, DGIST, Daegu, Republic of Korea  
e Core Protein Resources Center, DGIST, Daegu, Republic of Korea  
1 Department of Bio-Medical Analysis, Korea Polytechnic College, Chungnam, Republic of Korea  
f China-US (Henan) Hormel Cancer Institute, Zhengzhou, China  

** Corresponding author. Department of Animal Science and Biotechnology, ITRD, Kyungpook National University, Sangju, Republic of Korea  
** Corresponding author.  
E-mail addresses: jaewoong64@hanmail.net (Z.Y. Ryoo), ok4325@knu.ac.kr (M.O. Kim).  
1 Zhang and Yi equally contributed to this work.

1. Introduction

Colorectal cancer (CRC) was the third most common cancer diagnosed and the second leading cause of cancer-related deaths in the United States in 2020 [1]. Although significant progress has been made in multimodality therapy for CRC, the overall 5-yr survival rate remains poor [2]. Surgical resection and chemotherapy are common treatment options for patients with CRC; however, recurrence and distant metastases occurs frequently. Thus, new and more effective therapeutic targets and strategies are required.

Natural products have been used for disease treatment and prevention for thousands of years. Because of their relatively low toxicity, the anticancer activity of natural product compounds has attracted significant attention from researchers [3–5]. Ginseng is a well-known herbal medicine that exhibits various pharmacological and therapeutic activities. 20 (S)-ginsenoside Rh2 (G-Rh2) is one of

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the major active components of ginseng and shows potent anticancer effects in several cancer models [6–8]. G-Rh2 has been reported to suppress cervical cancer cell proliferation by inhibiting the AKT/GSK3β signaling pathway [9] and suppresses CRC cell growth by blocking the PKB/TOPK signaling pathway [7]. Moreover, G-Rh2 was reported to be effective at reversing drug resistance in several cancer types [10,11]. However, the effects and molecular mechanisms underlying G-Rh2 in CRC have yet to be fully elucidated.

Axl (also referred to as Ark, Tyro7, or Ufo) is a receptor tyrosine kinase that plays an important role in the metastatic potential and overall prognosis of many solid cancers [12–17]. Axl appears to function as an oncogene in various human malignancies, including pancreatic [18], breast [19], and lung [12,20] cancers. Axl functions through several downstream pathways including the MAPK/ERK, PI3K/AKT, and STAT3 signaling pathways depending on the cancer type [21–23]. BGB324 (R248) is a selective Axl inhibitor that has recently entered phase II clinical trials in multiple cancers [15,22]. These results indicate that Axl is an attractive therapeutic target for CRC treatment.

In this study, we found that G-Rh2 suppresses CRC cell growth by inhibiting the Axl signaling pathway in vitro and in vivo. Axl highly expressed in CRC cells and promotes the growth, migration, and invasion of CRC cells. Axl knockdown or treatment with G-Rh2 suppressed tumor growth in nude mice. Our findings indicate that G-Rh2 is a candidate for the prevention and treatment of CRC.

2. Materials and methods

2.1. Reagents

G-Rh2 (>98% purity) was obtained from Chengdu Biopurify Phytochemicals Ltd. (Chengdu, China). Primary antibodies for detecting p-Axl (Tyr702), VEGFR2, Src (32G6), p-Src (Tyr416), p-PI3K (Tyr458), p-PI3K, p-Akt (Ser473), Akt, p-ERK1/2, ERK1/2, cleaved-caspase3 (Asp175), cleaved PARP, survivin, CDK4, CDK6, p-PI3K (Tyr458), p-p38 (Thr180/Tyr182), p38, GSK-3β, p-GSK-3β, p-ERK1/2, ERK1/2, and Bax were purchased from Cell Signaling Technology (Beverly, MA, USA). Axl, Flt-1, and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Cell culture

Human CRC cell lines HCT15, HCT116, and DLD1 and the human colon fibroblast cell line CCD-18Co were purchased from the American Type Culture Collection (Manassas, VA, USA). HCT15 and DLD1 cells were grown in RPMI-1640 medium. HCT116 cells were grown in McCoy’s 5A medium. CCD-18Co cells were grown in MEM medium. All media contained 10% FBS (Gibco) and 1% penicillin/streptomycin. All the cells were maintained in a 37°C incubator with 5% CO2.

2.3. Cell viability assay

The Cell Counting Kit-8 (CCK-8; Dojindo, Japan) assays were used to measure cell viability. Briefly, cells were seeded into 96-well plates (1 × 10^4 cells/well) and exposed to varying concentrations of G-Rh2 for 0, 24, 48, and 72 h. At the corresponding time points, CCK-8 solution (10 μL) was added to each well of the plate and incubated for an additional 1 h at 37°C. The absorbance of each well was read at 450 nm using a spectrophotometer.

2.4. Anchorage-independent cell growth assay

CRC cells (8 × 10^3) were suspended in complete growth medium with 0.6% agar and various concentrations of G-Rh2 in the base layer and 0.3% agar with the various G-Rh2 concentrations in the top layer in 6-well plates. The plates were cultured in a cell culture incubator for 2 weeks. The number of colonies was subsequently counted using the ImageJ software.

2.5. Cell cycle distribution and apoptosis

Cells (2 × 10^5) were seeded in 60-mm dishes and exposed to different concentrations of G-Rh2 for 48 h. For cell cycle analysis, G-Rh2-treated cells were harvested and subsequently fixed in 70% ethanol overnight. The cells were then stained with propidium iodide (PI, 20 μg/mL). For apoptosis analysis, G-Rh2-treated cells were harvested and stained with annexin V (BioLegend, California, USA) and PI and analyzed by FACS Verse flow cytometry (BD Science, California, USA).

2.6. In vitro pull-down assay

CRC cell lysates (500 μg) were incubated with G-Rh2-Sepharose 4B (or Sepharose 4B only for the control) beads (50 μL, 50% slurry) in a reaction buffer (50 mM Tris pH 7.5, 5 mM EDTA, 150 mM NaCl, 1 mM DTT, 0.01% NP40, and 2 μg/mL bovine serum albumin) overnight at 4°C with gentle rocking. They were then washed five times with wash buffer (50 mM Tris pH 7.5, 5 mM EDTA, 150 mM NaCl, 1 mM DTT, and 0.01% NP40). Finally, protein binding was visualized by western blotting.

2.7. Immunofluorescence staining

CRC cells (1 × 10^5) were seeded into Lab-Tek II Chamber Slides (Thermo Fisher) and treated with different concentrations of G-Rh2 for 24 h. Cells were then fixed in 4% formaldehyde for 15 min. Following permeabilization with 0.3% Triton X-100, the cells were incubated with blocking buffer (5% bovine serum albumin in PBS) for 1 h followed by Axl antibody (1:200) overnight at 4°C. Subsequently, the cells were incubated with Alexa fluor 488-conjugated secondary antibody (Jackson ImmunoResearch Laboratories, Inc. West Grove, PA, USA) for 2 h at room temperature. After washing, the coverslips were mounted using a fluorescent mounting medium with 4',6-diamidino-2-phenylindole (DAPI). Representative images were captured using a fluorescence microscope (Leica).

2.8. Western blotting analysis

CRC cells (1 × 10^5) were seeded into 100-mm dishes and exposed to 0, 20, and 40 μM G-Rh2 at 37°C. After 48 h, cells were harvested and washed with ice-cold PBS on ice. Tumor tissues (50 mg) were crushed and ground into a powder with a liquid nitrogen-cooled mortar and pestle. Cells or tissues were lysed using PRO-PREP™ lysis buffer on ice for 40 min. The mixture was centrifuged and supernatants was collected. A total of 30 μg of protein was loaded and separated on SDS-PAGE gels (8%–12%) and transferred onto PVDF membranes (0.22 μm, Merck Millipore). The membranes were blocked using 5% BSA for 1 h and incubated with primary antibodies at 4°C overnight. The following day, the membranes were incubated with an HRP-conjugated secondary antibody for 1 h at room temperature. Signals were developed with an ECL detection kit and visualized with the Da Vinci Fluorescence Imaging System (Da Vinci-K, Seoul, Korea).
2.9. Transfection

The pcDNA Axl plasmid was a gift from Rosa Marina Melillo (Addgene plasmid #105932) [24]. The transfection experiments were carried out using FuGENE® HD transfection reagent (Promega) following the manufacturer’s instructions. To establish stable Axl-overexpressing cell lines, the transfected cell lines were exposed to 600 μg/mL of G418 (Invitrogen) for 2 wk. The selected cells were used in subsequent experiments.

2.10. Lentiviral production and infection

For Axl knockdown in CRC cells, five lentiviral human Axl shRNA vectors were purchased from Sigma-Aldrich company (SHCLND-NM_021913; among them sh-Axl#2 sequence: 5’-CCGGGCGGTCTGCATGAAGGAATTTCTCGAGAAATTCCTTCATGCAGA-3’, sh-Axl#5 sequence: 5’-CCGGGCGGTCTGCATGAAGGAATTTCTCGAGAAATTCCTTCATGCAGA-3’). Next, 293T cells were cotransfected with pLKO.1-scrambled, sh-Axl#2, and sh-Axl#5 vectors and packaging plasmids (pMD2.G, pMDL/g-p RRE, and pRSV-Rev) using the FuGENE® HD transfection reagent. After 48 h, the lentiviruses were harvested by filtration using a 0.45 μm filter. The cultured HCT116 cells were infected with lentiviruses using 8 μg/mL of polybrene (Sigma). After 24 h of infection, the medium was replaced with fresh complete growth medium containing 2 μg/mL of puromycin for 2 days. The selected cells were used for subsequent experiments.

2.11. In vivo xenograft model

BALB/c-nu mice (male, aged 5–6 weeks) were purchased from Orient Bio Inc. (Seongnam, Gyeonggi, Korea). All animal experiments were performed under protocols approved by and accordance with the guidelines of the Kyungpook National University Animal Use and Care Committee (2015-0135). HCT116 cells were collected in PBS at a final concentration of 1 × 10⁷ cells/mL. The cells (200 μL) were subcutaneously injected into the flanks of mice. Twelve days after injection, the mice were randomly divided into three groups (n = 6 mice/group) and treated with an intraperitoneal injection of G-Rh2 (10 mg/kg and 50 mg/kg) or vehicle (PBS) three times a week for 21 days. For Axl knockdown xenograft assays, the mice were randomly divided into two groups (n = 4 mice/group) and sh-Mock or sh-Axl#2 HCT116 cells (5 × 10⁶ cells in 0.2 mL PBS) were subcutaneously injected into the flanks of each mouse. Tumor volumes and body weights were recorded every 4 days. Tumor volumes were assessed using the following formula: 

\[ \text{Tumor volume} = \frac{4}{3} \pi \text{length} \times \text{width} \times \text{height} \]

2.12. Immunohistochemical analysis

Tissue samples from mice were fixed with formalin and embedded in a paraffin, and cut into 4-μm sections. Next, they were hybridized with primary antibodies Ki-67 (1:500), p-AKT (1:200), p-p38 (1:800), and Bax (1:200). Finally, the sections were mounted with DPX Mountant for histological analysis.

2.13. Statistical analysis

Statistical analysis was performed using the GraphPad Prism® software (GraphPad Software Inc. CA, USA). Statistically significant differences were tested using Student’s t-test. All data represent means ± SD from at least three individual experiments. P < 0.05 was considered statistically significant.

3. Results

3.1. G-Rh2 suppresses CRC cell growth without toxicity to normal colon fibroblast cells

To explore the anticancer activity of G-Rh2 in CRC cells, we investigated the effect of G-Rh2 on cellular response. We treated HCT15, HCT116, and DLD1 cells with 0, 10, 20, 40, or 60 μM G-Rh2 for 24 h in 6-well plates. After 24 h of treatment, we observed that CRC cell density significantly decreased compared with control cells at 40 μM G-Rh2 and most of the cells were dead at 60 μM (Fig. S1). Next, we used a CCK-8 assay to evaluate the cell viability of HCT15, HCT116, and DLD1 cells after treatment with various concentrations of G-Rh2 for 24 h. The results showed that G-Rh2 inhibited cell viability in a concentration-dependent manner and the IC₅₀ values for G-Rh2 on HCT15, HCT116, and DLD1 cells were 39.50, 40.81, and 46.16 μM, respectively (Fig. 1B). The viability of the CCD-18Co normal human colon fibroblast cell line was not affected by G-Rh2 treatment, even at 80 μM (Fig. 1B). Therefore, 0, 10, 20, and 40 μM of G-Rh2 were selected for additional in vitro studies. The CCK-8 assay results revealed that G-Rh2 significantly inhibited the proliferation of CRC cells in a dose- and time-dependent manner (Fig. 1C). Besides, G-Rh2 markedly reduced the number of colonies in an anchorage-independent cell growth assay (Fig. 1D and E). Collectively, these results indicate that G-Rh2 exhibits potent antitumor activity in CRC cells with minimal cytotoxic effects on normal colon fibroblast cells.

3.2. G-Rh2 inhibits the migration and invasion of CRC cells

To explore the effect of G-Rh2 on the migration and invasion capacity of CRC cells, wound-healing and transwell assays were conducted. The wound-healing assay results indicated that the migration of HCT15, HCT116, and DLD1 cells was significantly inhibited following treatment with G-Rh2 for 12 and 24 h compared with the control (Fig. 2A and B). Next, we performed transwell assays to further verify the effect of G-Rh2 on the cell migration and invasion ability. The transwell assay results indicated that G-Rh2 inhibited the migration and invasion capacity of CRC cells in a dose-dependent manner (Fig. 2C–F). Epithelial–mesenchymal transition (EMT) is known to be closely associated with cancer cell invasion and metastasis [25], and we detected the expression of EMT markers in CRC cells after G-Rh2 treatment by western blot analysis. Specifically, N-cadherin and vimentin were downregulated and E-cadherin was upregulated following treatment with G-Rh2 in HCT15, HCT116, and DLD1 cells (Fig. 2E). These results indicate that G-Rh2 plays a role in preventing CRC metastasis.

3.3. G-Rh2 induces G0/G1 phase arrest and apoptosis of CRC cells

To further explore the molecular mechanisms of G-Rh2 in CRC cell growth, we used a flow cytometry assay to determine cell cycle distribution and apoptosis in CRC cell lines following G-Rh2 treatment. As shown in Fig. 3A and B, compared with the control, G-Rh2 treatment significantly increased the ratio of G0/G1 phase cells. Then we explored how G-Rh2 regulated cell cycle-related proteins. We found that the G0/G1–phase cell cycle regulatory proteins, cyclin E1, cyclin D1, CDK 4, and CDK 6, were downregulated by G-Rh2 treatment in these three cell lines (Fig. 3E). In addition, the percentage of apoptotic HCT15, HCT116, and DLD1 cells significantly increased following treatment with G-Rh2 for 48 h (Fig. 3C and D). Moreover, G-Rh2 upregulated the protein levels of the proapoptotic proteins p53, cleaved caspase3, and cleaved PARP, whereas the
expression of the antiapoptotic protein survivin was down-regulated in CRC cells (Fig. 3F).

3.4. G-Rh2 directly binds to Axl and inhibits the Axl signaling pathway in CRC cells

Axl is a poor prognostic marker of colorectal cancer [26], and aberrant expression of Axl is related to cancer cell metastasis [27] and acquired drug resistance [20,28]. We found that the expression of p-Axl and Axl was higher in CRC cells (HCT15, HCT116, and DLD1) compared with CCD-18Co normal colon fibroblast cells (Fig. 4A). Some evidence indicated that G-Rh2 has a role in reversing drug resistance, such as Adriamycin-resistant breast cancer cells [29] and 5-FU resistance colorectal cancer cells [11]. Besides, G-Rh2 could regulate Axl downstream PI3K/Akt signaling pathways expression in several cancer types [30,31]. Thus, we considered whether G-Rh2 influences the Axl signaling pathway. To test this hypothesis, we performed an in vitro pull-down assay to explore whether G-Rh2 could bind with Axl proteins. The results indicated that G-Rh2 could directly bind to Axl (Fig. 4B). Besides, we found that G-Rh2 failed to bind with the receptor tyrosine kinases Flt-1 and VEGFR-2 (Fig. 4B). Then we detected the influence of G-Rh2 on Axl and its downstream protein expression by western blotting assay. We found that G-Rh2 inhibited the phosphorylation of Axl, Src, ERK, PI3K, AKT, mTOR, and GSK-3β in HCT15 and HCT116 cells (Fig. 4C). The immunofluorescence assays results showed that Axl expression was inhibited by G-Rh2 treatment in HCT15 and HCT116 cells (Fig. 4C). The immunofluorescence assays results showed that Axl expression was inhibited by G-Rh2 treatment in HCT15 and HCT116 cells (Fig. 4C). The immunofluorescence assays results showed that Axl expression was inhibited by G-Rh2 treatment in HCT15 and HCT116 cells (Fig. 4C). The immunofluorescence assays results showed that Axl expression was inhibited by G-Rh2 treatment in HCT15 and HCT116 cells (Fig. 4C). The immunofluorescence assays results showed that Axl expression was inhibited by G-Rh2 treatment in HCT15 and HCT116 cells (Fig. 4C). The immunofluorescence assays results showed that Axl expression was inhibited by G-Rh2 treatment in HCT15 and HCT116 cells (Fig. 4C).
Fig. 2. G-Rh2 suppresses the migration and invasion of CRC cells. (A) and (B) Wound-healing assay in HCT15, HCT116, and DLD1 cells following treatment with G-Rh2 for 12 and 24 h, respectively (scale bar, 250 μm). (C) and (D) The migration abilities of HCT15, HCT116, and DLD1 cells were determined by transwell assay after treatment with different concentrations of G-Rh2. (E) and (F) Invasion assay in HCT15, HCT116, and DLD1 cells following treatment with G-Rh2 for 24 h. (G) Expression of E-Cadherin, N-Cadherin, Vimentin, and β-actin in HCT15, HCT116, and DLD1 cells treated with different concentrations of G-Rh2.
3.5. Axl promotes the proliferation, migration, and invasion of CRC cells

To further investigate the functions of Axl in CRC cells, we knocked down Axl in HCT116 cells using lentiviral shRNA. Immunoblotting results showed that the Axl expression was silenced following infection with Axl shRNAs (especially sh-Axl#2 and sh-Axl#5) (Fig. 5A). Then, we explored the effect of Axl on CRC cell growth by various experiments. We found that the cell viability was significantly inhibited in Axl knockdown cells (Fig. 5B). In addition, Axl knockdown inhibited the cell migration and invasion ability (Fig. 5C and D) and induced Go/G1 phase cell cycle arrest (Fig. 5E and F). Knockdown of Axl decreased the colony number in HCT116 cells, whereas G-Rh2 failed to further inhibit colony formation in Axl knockdown cells compared with the control (Fig. 5G and H), indicating that Axl was the primary target of G-Rh2 during CRC cell growth. Our in vivo study showed that Axl knockdown significantly suppressed tumor growth and decreased tumor weight in xenograft mice (Fig. S4). In addition, to further verify the role of Axl in CRC cells, we established two stable Axl-overexpressing CRC cell lines (Fig. S5A). CCK-8 assays revealed that Axl overexpression significantly promoted CRC cell viability (Fig. S5B). Axl overexpression also increased the colony number in HCT15 and HCT116 cells (Figs S3C, S3D) and promoted the migration and invasion capacity of HCT15 and HCT116 cells (Figs S3E, S3F). Moreover, the overexpression of Axl diminished the effect of G-Rh2 in inhibiting the colony formation ability in HCT15 and HCT116 cells (Figs S3C, S3D). Collectively, these results indicate that Axl promotes the proliferation, migration, and invasion of CRC cells and that it is the main target of G-Rh2 to inhibit the growth of CRC cells.

3.6. G-Rh2 suppresses HCT116 xenograft tumor growth in nude mice

Based on our in vitro results, we further examined the antitumor effects of G-Rh2 in tumor-bearing mice. The xenograft model was established by subcutaneously injecting of HCT116 cells into the flanks of nude mice. After 12 days, the mice were divided into three groups, and treated with different doses of G-Rh2 for 3 weeks. The results indicated that 10 and 50 mg/kg of G-Rh2 significantly suppressed tumor growth compared with that in the vehicle-treated group (Fig. 6A and B). Meanwhile, G-Rh2 treatment reduced the protein levels of p-Axl, p-Src, p-ERK, and p-p38 in tumor tissues (Fig. 6D), which is consistent with our in vitro results. Additionally, there were no obvious differences in the histological structure of the liver and lung in these three groups, indicating that G-Rh2 was well-tolerated (Fig. 6C). Immunohistochemistry results indicated that the expression of Ki67, p-AKT, and p-p38 were significantly suppressed by G-Rh2 treatment, whereas the Bax expression was increased (Fig. 6E and F). These results indicate the HCT116 cell proliferation was inhibited and apoptosis was induced by G-Rh2 treatment in vivo. Collectively, G-Rh2 apparently inhibits HCT116 xenograft tumor growth in vivo by suppressing the Axl signaling pathway with no significant toxicity to mice. A schematic diagram for the underlying mechanism of the effects of G-Rh2 in CRC is shown in Fig. 6G.

4. Discussion

Colorectal cancer is still one of the cancers with a higher mortality rate, and it is necessary to find new treatment drugs. Accumulating evidence has indicated that many natural compounds can play a role in cancer prevention and treatment. G-Rh2 is an active component extracted from P. ginseng [32] that exerts potent anticancer activity in various cancers [7,33]. Previous studies indicated that G-Rh2 could inhibit CRC cell growth by activating p53 [34] and inhibiting TOPK activity [7]. In the present study, we found that G-Rh2 inhibits CRC cell growth by targeting and inhibiting the Axl signaling pathway in vitro and in vivo.

Axl has been reported to be highly expressed in several human cancers and associated with poor prognosis and drug resistance [15,35–38]. Axl is involved in cancer cell proliferation, migration, and invasion, rendering it a promising therapeutic target for cancer treatment [19,36]. Indeed, Axl has been shown to be a potential therapeutic target in HER2þ breast cancer [39] and lung cancer [40]. Suppressing Axl expression effectively inhibits tumor growth in various cancer models [41–43]. In this study, we found that Axl was highly expressed in CRC cells and G-Rh2 could directly bind to Axl, resulting in the inhibition of Axl signaling pathway. Axl knockdown suppressed CRC cell growth in vitro and in vivo, indicating that Axl plays an essential role in CRC development and progression. To verify the role of Axl in the process of G-Rh2 inhibiting the growth of CRC cells, we treated Axl overexpression and Axl knockdown cells with G-Rh2. We found that Axl overexpression can attenuate the effect of G-Rh2, whereas the growth inhibition effect of G-Rh2 in Axl knockdown cells was not further enhanced. This indicates that Axl is a key target of G-Rh2, which inhibits the growth of CRC cells.

PI3K/AKT is one of the downstream pathways of Axl, which plays a considerable role in many biological processes (including cell growth [44], cell cycle regulation [45], apoptosis [46]). Axl promotes tumor metastasis and decreases chemosensitivity through the activation of the PI3K/Akt/GSK3β signaling pathway [47]. Abnormal expressions of the PI3K/AKT pathway proteins are common in cancers including CRC [48]. Several PI3K/AKT signaling pathway inhibitors have exhibited antitumor effects in cancer treatment [49–51]. In this study, G-Rh2 suppressed the p-AKT, p-mTOR, and p-GSK-3β expressions by inhibiting Axl in CRC cells. Src, which is another downstream protein of Axl [52], was reported to mediate cell growth, migration, and angiogenesis through the MAPK, PI3K, and Stat3 signaling pathways [53,54]. Src can regulate cell proliferation and cell cycle arrest by activating ERK1/2 and AKT [55,56]. In our study, G-Rh2 significantly downregulated p-Src and p-ERK1/2 expressions in CRC cells both in vitro and in vivo.

EMT is a key process that influences tumor invasion and metastasis. A direct link between EMT and metastasis has been confirmed in various tumor cell lines [57]. Upregulation of N-cadherin and downregulation of E-cadherin is the hallmark of EMT in cancer cells [58]. We found that G-Rh2 significantly inhibited CRC cell migration and invasion in vitro by reducing the expression of vimentin and N-cadherin and increasing E-cadherin in CRC cells. This demonstrates that G-Rh2 suppresses EMT in CRC cells. A role for Axl in EMT has also been reported in various cancers. For example, one study reported that Axl upregulation controls the motility of breast cancer cells driven by EMT [59]. Axl is highly expressed in advanced CRC and was significantly related to the expression of cell migration genes [16]. Our results also indicate
that Axl is involved in the EMT process and that Axl overexpression promoted the migration and invasion ability of CRC cells, whereas its knockdown resulted in the opposite effect.

Animal models are widely regarded as essential for the study of the efficacy of antitumor drugs. G-Rh2 has been reported to inhibit tumors in several cancer xenograft models including H1299 lung...
Fig. 5. Axl knockdown inhibits CRC cell growth in vitro and in vivo. (A) Axl knockdown in HCT116 cells was detected by the western blotting. (B) Cell viability in Axl knockdown HCT116 cells was determined by the CCK-8 assay. (C) and (D) Migration and invasion ability of Axl knockdown HCT116 cells (scale bar, 128 µm). (E) and (F) Flow cytometry analysis of...
the cell cycle in Axl knockdown HCT116 cells. (G) and (H) Colony formation assay in Axl knockdown HCT116 cells treated with 0, 20, and 40 μM of G-Rh2 (scale bar, 250 μm). (I) Tumor volume. (J) Tumor weight. (K) Images of xenograft tumors. *P < 0.05, **P < 0.01, ***P < 0.001 versus control.
cancer cells [8] and A375 melanoma cells [60]. In this study, we investigated the antitumor effects of G-Rh2 in an HCT116-derived xenograft model and demonstrated that G-Rh2 significantly suppressed HCT116 xenograft tumor growth by inhibiting the Axl signaling pathway without obvious toxicity in nude mice.

In summary, our results indicate that G-Rh2 clearly inhibits CRC cell growth in vitro and in vivo by inhibiting the Axl signaling pathway. Our results indicate that G-Rh2 is a potential therapeutic candidate that should be further tested for use against CRC and other solid tumors.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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References


