Antimetastatic Effect of Salvicine on Human Breast Cancer MDA-MB-435 Orthotopic Xenograft Is Closely Related to Rho-Dependent Pathway

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Abstract

**Purpose:** Salvicine is a novel DNA topoisomerase II inhibitor with potent anticancer activity. In present study, the effect of salvicine against metastasis is evaluated using human breast carcinoma orthotopic metastasis model and its mechanism is further investigated both in animal and cellular levels.

**Experimental Design:** The MDA-MB-435 orthotopic xenograft model was applied to detect the antimetastatic effect of salvicine. Potential target candidates were detected and analyzed by microarray technology. Candidates were verified and explored by reverse transcription-PCR and Western blot. Salvicine activities on stress fiber formation, invasion, and membrane translocation were further investigated by immunofluorescence, invasion, and ultracentrifugal assays.

**Results:** Salvicine significantly reduced the lung metastatic foci of MDA-MB-435 orthotopic xenograft, without affecting primary tumor growth obviously. A comparison of gene expression profiles of primary tumors and lung metastatic focus between salvicine-treated and untreated groups using the CLOTECH Atlas human Cancer 1.2 cDNA microarray revealed that genes involved in tumor metastasis, particularly those closely related to cell adhesion and motility, were obviously down-regulated, including *fibronectin, integrin α3*, *integrin β3*, *integrin β5*, FAK, paxillin, and RhoC. Furthermore, salvicine significantly down-regulated RhoC at both mRNA and protein levels, greatly inhibited stress fiber formation and invasiveness of MDA-MB-435 cells, and markedly blocked translocation of both RhoA and RhoC from cytosol to membrane.

**Conclusion:** The unique antimetastatic action of salvicine, particularly its specific modulation of cell motility *in vivo* and *in vitro*, is closely related to Rho-dependent signaling pathway.

Metastasis refers to the dissemination of cancer cells from initial tumor to distant sites and involves a series of processes, including loss of adhesion, acquisition of cell motility, extracellular proteolysis, and angiogenesis (1). Cell motility plays a fundamental role in the onset and progression of cancer and is particularly important in tumor invasion and metastasis. Tumor cell lines that are more highly invasive and metastatic exhibit a higher degree of motility than their lower metastatic counterparts.

The dynamic organization of the actin cytoskeleton that provides the force for cell motility is regulated by Rho GTPases (2–4). The Rho GTPases, including Rac, cdc42, and Rho, are key regulators of the actin cytoskeleton (4). Rho, including RhoA, RhoB, and RhoC, controls actin stress fibers and focal adhesion contact formation, whereas Rac and cdc42 are responsible for the formation of lamellipodia and filopodia, respectively (4). Recent evidence shows that Rho is an essential regulator of cell motility and metastasis, (5–7) and its overexpression is intimately correlated with the invasive and/or metastatic phenotypes of numerous carcinomas (5, 7–17). Several upstream pathways that activate Rho as well as the downstream targets of activated Rho have been identified (18–22). Integrins, which directly or indirectly bind to talin, α-actinin, vinculin, paxillin, and focal adhesion kinase (FAK), mediated rearrangement of actin cytoskeleton in focal adhesion and focal adhesion complexes through a Rho-dependent pathway (23). Lysophosphatidic acid (LPA), a completely effective substitute for serum, (24) induces membrane translocation of Rho via specific G-protein–coupled receptors on the cell surface, which drives cytoskeletal contraction, leading to the activation of downstream effectors, and formation of focal adhesion and stress fibers promoting cell migration and invasion (25–28). Dominant inhibition of Rho function eliminates integrin clustering and LPA-induced migration and invasion activities *in vitro* (4, 29) and is markedly attributed to...
tumor metastasis reduction of mammary and lung carcinomas in vivo (5, 7, 29–31). Consistent with these results, agents targeting cell motility via in vivo inhibition of Rho/ROCK also result to substantial suppression of tumor metastasis without affecting the tumorigenicity (31–33). These findings suggest a potential therapeutic cure for tumor metastasis via blockage of Rho-dependent pathways (31, 34, 35).

Salvicine [4,5-seco-5,10-friedo-abiet-3,4-dihydroxy-5(10),6,8,13-tetraene-11,12-dione], a novel diterpenoid quinone compound, is a structurally modified derivative of a natural product lead from the traditional Chinese herb, Salvia prionitis (Labiatae). This compound is a novel DNA topoisomerase II inhibitor with evident anti-multiple drug-resistant activity, (36–38) and has entered a phase I clinical trial in China. Previous studies by our group highlighted the antiangiogenic potential of salvicine6 and prompted us to hypothesize that this compound may additionally exert anti-metastatic activity. Accordingly, in this study, the anti-metastatic effect of salvicine and its underlying molecular mechanisms of action were investigated at both the animal and cellular level.

Materials and Methods

Cell line. The human breast cancer MDA-MB-435 cell line was obtained from American Type Culture Collection (Rockville, MD). The culture was maintained in DMEM (Life Technologies, Inc., Grand Island, NY) supplemented with 10% fetal bovine serum, 2 mmol/L L-glutamine, 100 units/ml penicillin sodium, 100 μg/ml streptomycin sulfate, and 0.25 μg/ml amphotericin B (Life Technologies). Cells were cultured in a humidified atmosphere of 5% CO2 and 95% air at 37°C.

Animals. Female athymic nude mice (BALB/c nu/nu) ages 4 to 5 weeks were obtained from the Shanghai Institute of Materia Medica (Shanghai, China), housed in sterile cages under laminar airflow hoods in a specific pathogen-free room with a 12-hour light and 12-hour dark schedule and fed autoclaved chow and water ad libitum. All experiments were done according to institutional ethical guidelines on animal care.

Chemicals. Salvicine was structurally modified from the lead compound isolated from the Chinese medicinal plant S. prionitis by the Phytochemistry Department of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. The end product was purified from frozen tissue samples using Trizol (Life Technologies), total RNA or proteins extracted immediately.

RNA extraction and reverse transcription-PCR. Total RNA was extracted from frozen tissue samples using Trizol (Life Technologies), following the manufacturer’s instructions. The quantity and quality of RNA were assessed by spectrophotometry at 260 and 280 nm. Total RNA (1-3 μg) was subjected to reverse transcription. Next, cDNA was amplified using 2.5 units of Taq DNA Polymerase (Sino-American Biotechnology Co., Luoyang, China) and 0.2 μmol/L of specific oligonucleotide primers in a final reaction volume of 50 μL containing 10 mmol/LTris-HCl (pH 8.3), 50 mmol/L KCI, 2 mmol/L MgCl2, and 0.2 mmol/L each deoxynucleotide triphosphate (Sangon, Shanghai, China). The primers used in this experiment as following: sense 5′-CTGGTGATGGTTGCTAGTGG-3′ and antisense 5′-GGCATATAATCTTCCTGC-3′ for RhoA, 183 bp; sense 5′-ATGTGTCAGCAATCCGAAGAAG-3′ and antisense 5′-AGAAGGCAGGGGG-CATGTGCAGAAAAC-3′ for Adriaycin, 626 bp; sense 5′-ATGACCGCCTC-AAATGGTGTC-3′ for anti-sense 5′-5′ and antisense 5′-TACACGACAGG-CATTTCTCTCC-3′ for Rac1, 600 bp; sense 5′-CTCTCGTACTGAGTGCTAGG-3′ and antisense 5′-5′ for cd42, 250 bp; sense 5′-AAAGCTGACAGTGCAGAT-3′ and anti-sense 5′-AAAGATGATGATGATGATGATT-3′ for glyceraldehyde-3-phosphate dehydrogenase, 250 bp. Reverse transcription-PCR for target genes was done using a thermal cycler (MJ Research, Inc., Waltham, MA) according to a set program. The number of cycles was determined in preliminary experiments to be within the exponential range of PCR. Negative controls were run in parallel to confirm that samples were not contaminated with genomic DNA. PCR products (10 μL) were electrophoresed on a 2% agarose gel. Bands were visualized by ethidium bromide staining, and recorded using a UVG GDS8000 Gel Documentation System (UVP, Upland, CA).

Microarray analysis. We compared initial primary tumors versus metastases using the CLONTECH Atlas Human Cancer 1.2 CDNA microarray [1,176 genes]. Total RNA in tissue samples from three mice of each saline-treated or untreated group was isolated using Trizol reagent (Invitrogen, Carlsbad, CA), according to manufacturer’s instructions. Microarray analysis was done using the manufacturer’s protocol. Briefly, 1 μg total RNA was converted to 42P-labeled cDNA probes using Moloney murine leukemia virus reverse transcriptase and 42P-labeled cDNA probe was purified using CHROMA SPIN-200
Results

Salvicine suppresses lung metastasis of MDA-MB-435 orthotopic xenograft in athymic mice but does not affect primary tumor growth. We evaluated the efficacy of salvicine against tumor metastasis of the human breast cancer MDA-MB-435 orthotopic xenograft model in athymic mice. With weekly i.v. drug administration for 10 weeks, the number of pulmonary metastatic nodules of human breast cancer MDA-MB-435 orthotopic xenograft of control, etoposide 15 mg/kg, Adriamycin 5 mg/kg, salvinic 6 mg/kg, salvinic 12 mg/kg, and salvinic 24 mg/kg groups were 13.6 ± 4.8, 13.8 ± 8.3, 6.3 ± 1.5, 9.1 ± 3.9, 6.0 ± 4.2, and 2.5 ± 1.0, respectively. Salvicine (LD10ivw = 57.0 mg/kg) at doses of 6, 12, and 24 mg/kg significantly decreased lung metastasis of human breast cancer MDA-MB-435 orthotopic xenograft with inhibition rates of 33.0%, 54.0%, and 81.6%, respectively (Fig. 1A). However, salvicine did not noticeably affect primary tumor growth (T/C%) values at the last day of treatment in the first experiment were 122.7%, 162.4%, and 125.3%, respectively (Fig. 1B), and 115.3%, 82.0%, and 68.4%, respectively, in the second experiment (Fig. 1C). Etoposide, a DNA topoisomerase II inhibitor, given at a dose of 15 mg/kg (LD10ivw = 34 mg/kg), displayed no effect on tumor metastasis and proliferation (Fig. 1A and B). Adriamycin (5 mg/kg; LD10ivw = 10 mg/kg), another DNA topoisomerase II inhibitor, exhibited a 46.4% inhibitory effect against tumor metastasis (Fig. 1A), possibly due to its obvious blockage of primary tumor growth [T/C (%) at the last day of treatment was 17.4% (Fig. 1C)]. All mice treated with drugs survived with healthy appearance and no loss of body weight. The Rho-dependent signaling pathway is essentially involved in the in vivo antimetastatic efficacy of salvicine. The gene transcript expression profiling of primary and lung metastatic tumors of human breast cancer MDA-MB-435 orthotopic xenograft after salvicine treatment was evaluated with a CLONTECH Atlas human cancer 1.2 array containing 1,176 tumor-related genes. We set Min = 500, Max = 16,000, Max/Min ≥ 3, and Max-Min ≥ 100 as the filtering conditions. Following normalization and filtering, 872 genes were further analyzed with Genecluster 2.0 software by self-organized mapping, unsupervised hierarchical clustering, and function classification analysis. Based on 2× self-organized mapping analysis, all groups were divided automatically into two clusters. In cluster 0, the metastatic tumor groups treated with salvicine (6, 12, and 24 mg/kg) displayed distance/similarity values of 0.041, 0.074, and 0.83, respectively, observed in negatively controlled primary tumor group, 12 mg/kg salvinic-treated primary tumor group, 24 mg/kg...
salvicine-treated primary tumor group, and negatively controlled metastatic tumor group were 0.044, 0.050, 0.033, and 0.035, respectively (Fig. 2). Little or no differences were observed between the negatively controlled metastatic tumor group and the negatively controlled primary tumor group. The results indicate that gene expression profiles of metastatic tumors were significantly affected by salvicine, whereas those of the primary tumor were less affected.

Expressed genes were further grouped by detailed application of CLONTECH gene function classification analysis. Salvicine altered the expression of 14 in 125 genes implicated in the cell cycle (11.2%; e.g., increased expression of cyclin 1, CDK3, CDK9, MTA-1, and cdc-like kinase 2 and decreased the expression of growth inhibitory factor, CDK6, G1-S transition protein 1, and PRL-1). The compound additionally altered the levels of 4 in 66 genes involved in DNA synthesis and damage repair (6.1%; e.g., increased expression of BRCA2 and PCNA and decreased expression of GSH and MSH6). Importantly, salvicine affected the expression of 15 of 80 genes related to cell motility (18.8%; e.g., up-regulated tight junction protein 1, RAP1A, N-Ras, RabB, cdc42 homologue and down-regulated cytokeratin 8, cytokeratin 12, BIGH3, RhoC, Rac1, and motility-related protein) and influenced 13 in 64 genes implicated in cell adhesion (20.3%; e.g., increased expression of cadherin 4, plexin-related protein and decreased expression of Integrin z3, Integrin z6, Integrin z, Integrin b3, Integrin b5, Integrin b8, paxillin, and FAK). Moreover, treatment with salvicine reduced the expression of 14 in 86 genes involved in proteolysis and extracellular matrix proteins (16.3%; e.g., collagen 8a1, collagen 16a1, fibronectin, tenascin, osteonectin, MMP11, TIMP1, TIMP3, t-PA). Those results show that genes involved in tumor metastasis are strongly influenced by salvicine.

We further analyzed those genes by the properties of tumor metastasis, including cell adhesion, motility, proteolysis, and angiogenesis. It was found that salvicine greatly influenced the mRNA expression of genes implicated in cell adhesion, such as fibronectin, osteonectin, Integrin z3, Integrin z6, Integrin z, Integrin b3, Integrin b5, Integrin b8, paxillin, and FAK and markedly reduced the transcript expression of cell motility-related genes, including cytokeratin 8, cytokeratin 12, BIGH3,
Salvicine disrupts Rho-induced stress fiber formation. LPA is a serum phospholipid with growth factor–like activities for many cell types, expressed with significant levels (>1 μmol/L) in various human body fluids. LPA can activate Rho function, trigger cytoskeletal reorganization, induce stress fiber formation, and alter cell morphology. Accordingly, we determined whether salvicine affects Rho-induced stress fiber formation of human breast cancer MDA-MB-435 cells with or without specific Rho activator stimulation. As shown in Fig. 4A, serum-starved cells showed low levels of stress fibers, as evaluated from the Texas Red-X phalloidin (combining filamentous actin) immunostaining assay (Fig. 4A, a). Typical shapes of MDA-MB-435 cells cultured in DMEM with 10% fetal bovine serum were flat, well spread, and rich in stress fibers (Fig. 4A, b). In contrast, exposure of MDA-MB-435 cells to 10 μmol/L LPA for 10 minutes induced a dramatic increase in stress fiber formation (Fig. 4A, c). Importantly, treatment of 15 μmol/L salvicine for 16 hours resulted in an obvious reduction of stress fiber formation in LPA-stimulated MDA-MB-435 cells, accompanied by cell retraction from the substratum, rounding up, and loss of contact between neighboring cells (Fig. 4A, i). In addition, salvicine also markedly disrupted the formation of stress fibers in serum-stimulated MDA-MB-435 cells (Fig. 4A, h). C3 exoenzyme, a Rho-specific inhibitor (50 μg/mL) displayed similar results as salvicine, regardless LPA treatment, whereas Y27632, a ROCK-specific inhibitor (20 μmol/L), induced reduction in stress fiber formation, but didn’t change the elongated cell shape (Fig. 4A, d, e, f, g).

Salvicine antagonizes the lysophosphatidic acid–induced in vitro invasive capacity of human breast cancer MDA-MB-435 cells. Next, we designed an experiment to determine the effect of salvicine on the in vitro invasion capacity stimulated by LPA or serum using the Matrigel invasion assay. In the presence of 10 μmol/L LPA, the invasion capacity of MDA-MB-435 cells was increased to ~200% (Fig. 5A and B). Migration of MDA-MB-435 cells through the Matrigel-coated polycarbonate membrane was inhibited by salvicine in a dose-dependent manner, with half-maximal inhibition at about 7.5 μmol/L, and ~100% inhibition at 20 μmol/L. C3 exoenzyme 50 μg/mL also displayed inhibitory effects (Fig. 5A and B). In addition, salvicine, C3 exoenzyme, and Y27632 showed similar inhibitory effects against the in vitro invasiveness of 20% FCS-stimulated MDA-MB-435 cells (data not shown). Interestingly, 20 μmol/L salvicine did not block MDA-MB-435 cell attachment to Matrigel-coated membranes 4 hours after seeding or affect cell growth during the 20 hours of incubation period. Our results confirm that salvicine inhibits the Rho-activated migration of cells towards the chemoattractant, LPA, which is located in the lower chamber.

Salvicine blocks lysophosphatidic acid–induced translocation of RhoA and RhoC from the cytosol to membrane. Rho proteins must be targeted to the plasma membrane for activation and full function, which depends on lipid modification. LPA can promote the translocation of Rho from cytosol to the membrane via G12/G13 proteins; thus, we detected whether salvicine exerts the inhibitory effect against translocation of RhoA and RhoC from cytosol to membrane. In subcellular localization assay, Rho protein was mainly distributed in the cell cytosol in serum-starved condition and greatly clustered in cell membrane after LPA stimulation (Fig. 4B). Salvicine effectively blocked LPA-stimulated translocation of both RhoA and RhoC from cytosol to membrane in MDA-MB-435 cells (Fig. 4B). In membrane protein isolation experiment, it was revealed that stimulation of MDA-MB-435 cells with 10 μmol/L LPA for 10 minutes led to a dramatic increase in the amount of RhoA and RhoC in the membrane fraction, compared with control (Fig. 4C and D). Salvicine inhibited LPA-induced cell membrane translocation of RhoA and RhoC significantly in a time- and dose-dependent manner but had no obvious influence on the translocation of G12 and G13 (Fig. 4C and D). Half-maximal inhibition by salvicine was estimated at about 7.5 μmol/L for 16 hours or about 20 μmol/L at <8 hours. Additionally, treatment with salvicine 20 μmol/L for 16 hours led to a less expression of active RhoA and RhoC (in the membrane fraction) than that of the nonstimulated group (Fig. 4C and D).
The panel of antitumor drugs, mostly discovered by chance or semiempirical procedures, is largely inefficient for treating disseminate diseases, and there is currently a pressing need for antimetastasis drugs. “True” antimetastatic agents should only include those compounds that repeatedly display the capacity to selectively interfere with metastasis formation, with marginal or no effect on primary tumor growth (40). To elucidate mechanisms of those compounds will be great helpful for understanding metastasis itself and finding potential targets for metastasis prevention.

In the present study, we show that the topoisomerase II inhibitor salvicine reduces the number of pulmonary metastatic colonies of human breast cancer MDA-MB-435 orthotopic xenograft in a dose-dependent manner without obviously affecting primary tumor growth. However, two other topoisomerase II inhibitors, Adriamycin and etoposide, display different modes of action. Etoposide fails to combat tumor metastasis, whereas Adriamycin suppresses tumor

### Table 1. The gene expression profiles of primary and metastatic tumors of MDA-MB-435 orthotopic xenografts after salvicine treatment in vivo

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<th>Function</th>
<th>Genes</th>
<th>Primary tumor salvinic, 12 mg/kg</th>
<th>Primary tumor salvinic, 24 mg/kg</th>
<th>Lung metastatic foci salvinic, 6 mg/kg</th>
<th>Lung metastatic foci salvinic, 12 mg/kg</th>
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**NOTE:** Data are expressed as the ratio of salvicine-treated groups versus untreated groups in primary and metastatic tumors respectively in a typical experiment. Similar results were obtained from at least three separate experiments. When the ratio was >1.5 or <0.75, it was significant. It was regarded as not significant (NS) when the difference of minimum value and maximum value of gene expression is <500.
metastasis, which may be attributable to its marked inhibition of primary tumor growth. In view of these findings, we propose that salvicine combats tumor metastasis via a unique mechanism distinguishable from that of antitumor cytotoxicity.

In the following study, we compare transcript expression profilings of primary and metastatic tumors after salvicine treatment to find potential targets involved in its antimetastasis processes. The self-organized mapping and gene function classification analysis indicate significant influence of salvicine on genes involved in tumor metastasis. To further dissect the signal pathway mainly influenced by salvicine treatment, we find that components of Integrin-Rho pathway are mostly affected in the extent and range, such as integrin α6, integrin β3, integrin β5, fibronectin (integrin ligand), FAK, paxillin, and RhoC. Integrins play an important role in organizing the actin cytoskeleton at sites of adhesion to the extracellular matrix, such as focal complexes and focal adhesions. Integrins, which directly or indirectly bind to talin, α-actinin, vinculin, paxillin, and FAK, mediated rearrangement of actin cytoskeleton in focal adhesion and focal adhesion complexes through a Rho-dependent pathway (23).

In the present study, we show that salvicine greatly disrupts Rho-induced stress fiber formation and blocks the in vitro invasiveness, accompanied by the inhibition of RhoC at both the protein and mRNA levels in MDA-MB-435 cells. In addition, we also observe that salvicine inhibits tyrosine phosphorylation of FAK and paxillin in fibronectin-stimulated MDA-MB-435 cells. Because inhibition or loss of components of Integrin-Rho pathway (e.g., dominant-negative Rho or Rho inhibitors) severely restricts adhesion complex turnover and inhibits cell motility, (18, 41–44); thus,

down-regulated expressions of genes in this pathway, such as integrins, fibronectin, FAK, paxillin, and RhoC, all contributed to blockage of cell motility and tumor metastasis by salvicine treatment. These findings also provide a reasonable explanation for the suppression of tumor metastasis by salvicine in vivo.

LPA is a serum phospholipid with growth factor–like activities for many cell types, expressed with significant levels (>1 μmol/L) in various human body fluids (25). Its aberrant expression and signaling probable contribute to cancer initiation, progression and metastasis. LPA can promote Rho translocation from cytosol to the membrane via specific G-protein–coupled receptors on the cell surface, leading to activation of many downstream effectors and formation of cell adhesion and stress fibers, and plays a dominant role in tumor cell migration and invasion (25). The membrane translocation of Rho GTPases, which maybe required for ATP ribosylation, (45) is necessary to trigger cascades that lead to their subsequent full function (46). In the present study, salvicine significantly blocks the LPA-induced translocation of RhoA and RhoC from cytosol to the membrane fraction in a time- and dose-dependent manner in LPA-stimulated MDA-MB-435 cells, detected by both subcellular localization immunofluorescence staining and ultracentrifugal membrane protein isolation assays. It also markedly disrupts Rho-induced stress fiber formation and blocks the in vitro invasiveness of LPA-stimulated MDA-MB-435 cells, characterized as the blockage of the translocation of RhoA

6 Unpublished data.
and RhoC from the cytosol to the membrane. The blockage of membrane translocation of Rho leads to significant reduction of stress fiber formation and invasion induced by integrin clustering and LPA (29, 46–48). Cumulatively, the data imply that inhibition of Rho translocation from cytosol to membrane plays important role in salvicine’s antimetastasis processes in vivo. In addition, LPA can promote the Integrin-Rho signaling via the positive feedback loop between integrins and Rho GTPases, leading to clustering of integrins, tyrosine phosphorylation of FAK and paxillin, and formation of focal adhesions and stress fibers (44, 49). Inhibition of Rho function can also block LPA-induced integrin clustering, phosphorylation of FAK and paxillin, and stress fibers formation (26, 29). Taken together, it signifies the strict involvement of Rho-dependent signaling pathways in regulating antimetastatic processes of salvicine.

The C3 exoenzyme from Clostridium botulinum, the prototype protein for this family of toxins, was the first bacterial toxin shown to catalyze covalent modification of a Rho GTPases (33). The enzyme efficiently ADP-ribosylates RhoA, RhoB, and RhoC, with marginal or no modification of Rac or cdc42, and often serves as a useful tool to characterize the pharmacologic and cell biological functions of Rho Proteins (33). The biological changes after salvicine treatment, such as cell retraction from the substratum, rounding up, loss of contacts between neighboring cells, stress fiber reduction, and blockage of invasiveness, were similar as that of C3 in MDA-MB-435 cells.

Having established that the antimetastatic activities of salvicine correlate with cell membrane translocation of the Rho protein family, we further attempted to characterize the detailed regulatory mechanisms. Recent exciting evidence shows that LPA-stimulated translocation processes of RhoA and RhoC are regulated by G12/13, major upstream regulators of Rho function. In contrast to the positive effects of LPA on G12/13 expression, salvicine had a negative effect. This finding indicates that the antagonizing potency of salvicine against cell membrane translocation is independent of G12/13, and the compound might affect other downstream effectors, such as Rho guanine nucleotide exchange factors. Related studies on Rho guanine nucleotide exchange factors and isoprenylation are currently under way.

It is also interesting to investigate how salvicine affects the expression of Rho proteins. The three isoforms of Rho share 85% amino acid sequence identity, but in the present study, we found that salvicine inhibited the expression of RhoC, whereas not affecting RhoA. It might be due to that RhoA, RhoB, and RhoC had preferential interactions with different regulators.
and effectors in signal transduction context, and their gene expression depended on tissue types very significantly and were regulated by different mechanism (50).

In conclusion, this is the first study to disclose a unique underlying mechanism of salvicine against tumor metastasis, which is one of few compounds interfering with metastasis formation with marginal or no effect on primary tumor growth. The inhibition of Rho-dependent signaling pathways is at least partially responsible for salvicine’s antimetastatic effects, both in vitro and in vivo. These findings support that Rho-dependent pathway, particularly Rho, might be a therapeutic potential target for tumor metastasis cure. Careful elucidation of the molecular mechanisms underlying the antimetastatic action of salvicine may widen its clinical applications, and provide novel structural data for targeting Rho-associated proteins in anti-metastasis drug development.

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