Isorhamnetin Suppresses Skin Cancer through Direct Inhibition of MEK1 and PI3-K

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Abstract

3'-Methoxy-3,4',5,7-tetrahydroxyflavone (isorhamnetin) is a plant flavonoid that occurs in fruits and medicinal herbs. Isorhamnetin exerts anticancer effects, but the underlying molecular mechanism for the chemopreventive potential of isorhamnetin remains unknown. Here, we report anti–skin cancer effects of isorhamnetin, which inhibited epidermal growth factor (EGF)-induced neoplastic cell transformation. It also suppressed anchorage-dependent and -independent growth of A431 human epithelial carcinoma cells. Isorhamnetin attenuated EGF-induced COX-2 expression in JB6 and A431 cells. In an in vivo mouse xenograft using A431 cells, isorhamnetin reduced tumor growth and COX-2 expression. The EGF-induced phosphorylation of extracellular signal-regulated kinases, p90 and p70 ribosomal S6 kinases, and Akt was suppressed by isorhamnetin. In vitro and ex vivo kinase assay data showed that isorhamnetin inhibited the kinase activity of MAP (mitogen-activated protein)/ERK (extracellular signal regulated kinase) kinase (MEK) 1 and PI3-K (phosphoinositide 3-kinase) and the inhibition was due to direct binding with isorhamnetin. Notably, isorhamnetin bound directly to MEK1 in an ATP-noncompetitive manner and to PI3-K in an ATP-competitive manner. This report is the first mechanistic study identifying a clear molecular target for the anticancer activity of isorhamnetin. Overall, these results indicate that isorhamnetin has potent anticancer activity and it primarily targets MEK and PI3-K, which might contribute to the chemopreventive potential of certain foods. Cancer Prev Res; 4(4); 582–91. ©2011 AACR.

Introduction

Nonmelanoma skin cancer (NMSC) is the most commonly diagnosed cancer in the United States. About 1.3 million individuals suffer from this disease (1). The most common types of NMSC are basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), which develop from basal cells and keratinocytes, respectively. BCC comprises 75% of all NMSC cases and SCC makes up 20%. SCC, but not BCC, can metastasize to other areas of the body (2). Because NMSC is readily detectable and has limited malignancy, the mortality of NMSC is low compared with other cancers (3). However, because of its high incidence, NMSC is the fifth most costly cancer in the United States. Medicare spends $13 billion each year on skin cancer treatment (4). Changing environmental conditions, such as the destruction of the ozone layer and environmental pollution, and alterations in eating habits and increased longevity enhance the incidence of NMSC (5). A suitable chemopreventive agent could prevent and cure NMSC, which would help people avoid the pain of skin cancer and reduce Medicare costs (6).

UV radiation is the most important cause of skin cancer and chronic illnesses, and carcinogenic chemical exposures are also major factors (1). These causes of skin cancer activate multiple cellular signaling pathways and convert normal cells to cancerous cells. An important mediator of these signaling pathways is the epidermal growth factor (EGF) receptor (EGFR), which belongs to the ErbB family of receptor tyrosine kinases (RTK). This receptor is dimerized by binding a ligand, such as EGF (7), which activates the intercellular tyrosine kinase domain. The activated EGFR tyrosine kinase domain activates the mitogen-activated protein (MAP) kinase and phosphoinositide 3-kinase (PI3-K) (ref. 8). Previous studies have shown that...
the EGFR is required for UV-induced NMSC development (9). Chemical carcinogens, such as arsenite, also activate EGFR signal transduction. Both SCC and BCC highly overexpress the EGFR (10). Thus, a compound that targets the EGFR signaling pathway might be useful in NMCS prevention and treatment.

COX-2 plays a crucial role in inflammation, tumorigenesis, and angiogenesis. It is overexpressed in many types of cancer. The development of intestinal tumors and skin papillomas were reportedly reduced in a COX-2 knockout mouse model (11). The pharmacologic inhibition of COX-2 also impairs tumor formation in many animal models (12), and COX-2 is apparently required for neoplastic transformation (13). Thus, the inhibition of COX-2 expression might also be a promising target for preventing carcinogenesis.

3′-Methoxy-3,4′,5,7-tetrahydroxyflavone (i.e., isorhamnetin) is an active compound in herbal medicinal plants, such as Persicaria thunbergii H. and gross Hippophae rhamnoides L., and it has been used to treat cardiovascular disease, rheumatism, and hemorrhage (14–16). Recent studies have shown that isorhamnetin exerts anticancer effects. I sorhamnetin inhibits the proliferation of several cancer cell lines and suppresses the weight and size of tumors of Lewis lung carcinoma cell allografts in mice (15, 17, 18). However, the inhibitory effect of isorhamnetin on skin cancer and the underlying molecular mechanisms are not fully understood. In this study, we examined the inhibitory effects of isorhamnetin against skin cancer and suggest that MAP/ERK (extracellular signal regulated kinase) kinase (MEK) 1 and PI3-K as novel targets of isorhamnetin.

Materials and Methods

Chemicals and reagents

isorhamnetin was purchased from Sigma Aldrich and its purity is more than 95%. EGF was purchased from R&D Systems. Antibodies were from Cell Signal Biotechnology and Santa Cruz Biotechnology. The MEK1 and PI3-K assay kits were obtained from Millipore Corporation. Other chemicals were from Sigma-Aldrich.

Western blot analysis

Western blot analyses were conducted as described in our previous studies (19).

Anchorage-independent cell transformation assay

This assay was conducted as reported in our previous studies (19).

Cell viability

Cell viability was assessed as explained in our previous studies (19).

Xenograft study

Female athymic nude mice were purchased from Orient. Each animal was injected subcutaneously in the flank with A431 cells (1×10^6 cells in 50 µL of medium and 50 µL of Matrigel). Cells were allowed to form tumors, and once the tumors reached a size of 40 mm^3, the mice were randomly assigned into groups (6 mice/group) and treated with (1 or 5 mg/kg body weight) or without isorhamnetin in 40% DMSO/PBS buffer, administered intraperitoneally every other day for 28 days. Tumor size was measured every week with calipers, and the tumor volume was calculated according to a standard formula: width × length × height/2. Mice were sacrificed after 28 days of treatment when the control tumors reached approximately 600 mm^3. The tumors were harvested, photographed, and weighed. Tumor tissues were used for Western blot analysis and immunohistochemical analysis.

Immunohistochemistry

For an immunohistochemical analysis of COX-2 protein expression, excised tumors were fixed in 10% formalin for 1 day, embedded in paraffin, and cut into 5-µm-thick sections. Serial sections were mounted on Silane-coated slides, deparaffinized 3× with xylene, and dehydrated through a gradient alcohol series. The deparaffinized sections were boiled in 0.01 mol/L citrate buffer (pH 6.0) for 15 minutes for antigen retrieval. Sections were washed in PBST (PBS with Tween 20) and placed in blocking buffer for 30 minutes, followed by incubation with a primary antibody against COX-2 (1:50 dilution) for 12 hours at 4°C. Next, the endogenous peroxidase activity of paraffin-embedded sections was treated with 3% hydrogen peroxide for 10 minutes in blocking solution, consisting of PBS with 1% bovine serum albumin (BSA). The slides were then incubated with anti-rabbit horseradish peroxidase–conjugated secondary antibody for 30 minutes. The immunoreactive complexes were detected by staining with 3,3′-diaminobenzidine tetrahydrochloride hydrate and counterstained with Mayer’s hematoxylin.

Luciferase assays for COX-2 promoter activity and AP-1 transcription activity

The luciferase activity was determined as previously reported (19).

Kinase assays

Kinase assays were conducted as reported in our previous studies (19, 20).

Direct and cell-based pull-down assays

Active MEK1 protein, PI3-K (200 ng), or a JB6 cellular supernatant fraction (500 µg) was incubated with isorhamnetin-Sepharose 4B or Sepharose 4B beads alone as a control (100 µL, 50% slurry) in reaction buffer (50 mmol/L Tris, pH 7.5, 5 mmol/L EDTA, 150 mmol/L NaCl, 1 mmol/L DTT, 0.01% Nonidet P-40, 2 µg/µL BSA, 0.02 mol/L phenylmethylsulfonyl fluoride (PMSF), and 1× protease inhibitor mixture). After incubation with gentle rocking overnight at 4°C, the beads were washed 5× with reaction buffer (50 mmol/L Tris, pH 7.5, 5 mmol/L EDTA, 150 mmol/L NaCl, 1 mmol/L DTT, 0.01% Nonidet P-40,
and 0.02 mmol/L PMSF) and proteins bound to the beads were analyzed by Western blotting.

**ATP and isorhamnetin competition assay**

Recombinant MEK1 or PI3-K (0.2 μg) was incubated with 100 μL of isorhamnetin-Sepharose 4B or 100 μL of Sepharose 4B beads in reaction buffer (see the previous text) for 12 hours at 4°C, and ATP was added at either 10 or 100 μmol/L to a final volume of 500 μL, followed by incubation for 30 minutes. The samples were washed and proteins were detected by Western blotting.

**Molecular modeling**

Insight II (Accelrys Inc.) was utilized for the docking study and structural analysis by using the crystal coordinates for MEK1 (PDB code 1S9J) and PI3-K complexed with ATP or quercetin (1E8X or 1E8W), available from the Protein Data Bank (http://www.rcsb.org/pdb/).

**Statistical analysis**

When necessary, data are expressed as means ± SD and a one-way ANOVA was used for single statistical comparisons. A probability value of $P < 0.05$ was used as the criterion for significance.

**Results**

**Isorhamnetin strongly inhibits EGF-induced neoplastic transformation of JB6 cells and anchorage-dependent and-independent cell growth of A431 cells**

We first examined the effects of isorhamnetin on EGF-induced neoplastic transformation in JB6 cells. On the basis of the numbers of cell colonies, EGF-promoted neoplastic transformation was markedly inhibited in JB6 cells treated with 10 μmol/L isorhamnetin (Fig. 1A and B). Isorhamnetin concentrations between 10 and 40 μmol/L had no effect on cell viability after 3 days (Supplementary Fig. S1), suggesting that isorhamnetin inhibits EGF-induced neoplastic transformation without affecting viability of JB6 cells. Because A431 cells overexpress EGFR and can grow in athymic nude mice, this is an excellent model for studying EGF-mediated skin cancer (21). We measured anchorage-dependent and -independent cell growth of A431 cells by MTS assay and a soft agar assay, respectively.

![Figure 1](https://cancerpreventionresearch.aacrjournals.org)
Isorhamnetin suppressed both anchorage-dependent and -independent growth of A431 cells (Fig. 1C and D).

**Isorhamnetin suppresses COX-2 protein expression in JB6 and A431 cells**

Because COX-2 is one of the most important inflammatory mediators of skin cancer (22), we examined the effects of isorhamnetin on EGF-induced COX-2 expression in JB6 cells. COX-2 expression was inhibited dose-dependently by isorhamnetin treatment (Fig. 2A). To determine whether the inhibitory effects of isorhamnetin on COX-2 expression are mediated by transcriptional regulation, we investigated the effects of isorhamnetin on EGF-induced COX-2 promoter activity. The luciferase assay revealed that exposure to EGF (10 ng/mL) significantly induced COX-2 promoter activity, and it was suppressed by isorhamnetin treatment in a dose-dependent manner (Fig. 2B). JB6 cells stably transfected with an AP-1 luciferase reporter plasmid were used to measure AP-1 transactivation, which is a well-known transcription factor regulating COX-2 expression. Isorhamnetin inhibited EGF-induced AP-1 transactivation (Supplementary Fig. S2) in a dose-dependent manner. Next, we examined the effect of isorhamnetin on COX-2 protein expression in A431 cells and found that after 12 hours of treatment with 40 μmol/L isorhamnetin, COX-2 expression had completely disappeared (Fig. 2C). Isorhamnetin at 10 μmol/L also suppressed COX-2 expression completely in A431 cells (Fig. 2D).

**Isorhamnetin suppresses growth and COX-2 expression in A431 xenograft tumors in nude mice**

Because isorhamnetin was observed to be effective in suppressing the proliferation of A431 cells, we studied the effects of isorhamnetin in an *in vivo* xenograft model to further confirm the antitumorigenic activity of isorhamnetin. No significant body weight loss or appearance change was observed in mice treated with isorhamnetin compared with controls, indicating that the doses used were not toxic to the animals (Supplementary Fig. S3). Photographic data showed that isorhamnetin treatment suppressed tumor development in mice (Fig. 3A). The average volume of tumors in untreated mice increased over time and reached a volume of 623 mm$^3$ at 4 weeks postinoculation; however, at this time, in mice treated with 1 or 5 mg/kg isorhamnetin, the average tumor volume was only 280 or 198 mm$^3$, respectively (Fig. 3B). At the end of the study, we removed...
and weighed the tumors for each group. Clearly, isorhamnetin treatment (1 or 5 mg/kg) reduced tumor weight compared with the untreated control group (Fig. 3C). Collectively, these results suggest that isorhamnetin might serve as an effective anticancer treatment with the potential to suppress or delay the tumorigenicity of A431 cells in an in vivo system. To further confirm the inhibitory effect of isorhamnetin on COX-2 protein expression in xenograft tumors in athymic nude mice. The immunostaining data are representative of 3 tissue samples from each group and COX-2 appears brown. Arrows indicate the regions exhibiting immunoreactivity for COX-2 proteins.

Isorhamnetin inhibits the phosphorylation of ERKs and Akt in JB6 and A431 cells

To examine the mechanism of the inhibitory effects of isorhamnetin on EGF-induced cell transformation and COX-2 protein expression, we examined the effects of isorhamnetin on the ERKs and PI3-K signaling pathways, which play key roles in EGFR-mediated cell growth and COX-2 expression (19, 23). Exposure of JB6 cells to EGF (10 ng/mL) markedly induced the phosphorylation of ERKs and Akt. Isorhamnetin suppressed ERK/p90RSK but not MEK1/2 (Fig. 4A) and also inhibited the phosphorylation of Akt/p70S6K (Fig. 4B). Next, we investigated the change in phosphorylation status in A431 cells by isorhamnetin treatment in a time- and concentration-dependent manner. As for JB6 cells, isorhamnetin suppressed the phosphorylation of ERKs and p90RSK but not MEK and also inhibited AKT/p70S6K (Fig. 4C and D, Supplemental Fig. S4).

Isorhamnetin binds to and directly inhibits MEK1 and PI3-K activities

To examine the mechanism of isorhamnetin inhibition of the EGF-induced signaling pathway, we searched for the molecular target of isorhamnetin. Because isorhamnetin suppressed EGF-induced phosphorylation of ERKs, but increased MEK1/2 phosphorylation, we measured the
effect of isorhamnetin on MEK1 kinase activity. Data from our *in vitro* kinase assays indicated that isorhamnetin strongly inhibited MEK1 kinase activity [Fig. 5A (a)] and also suppressed EGF-induced MEK1 activity [Fig. 5A (b)]. Because isorhamnetin attenuated the EGF-induced Akt/ p70S6K pathway, and PI3-K is an upstream kinase of Akt, we investigated whether PI3-K was another molecular target of isorhamnetin. We found that isorhamnetin inhibited PI3-K *in vitro* [Fig. 5B (a)] and *ex vivo* [Fig. 5B (b)].

Next, we investigated whether isorhamnetin interacted directly with MEK1 or PI3-K. The direct binding of isorhamnetin to MEK1 or PI3-K was shown by an *in vitro* pull-down assay [Fig. 5C (a) and D (a)]. In addition, we observed *ex vivo* binding between isorhamnetin and MEK1 or PI3-K in JB6 cell lysates [Fig. 5C (b) and D (b)].

**Isorhamnetin binds to MEK1 in an ATP-noncompetitive manner and to PI3-K in an ATP-competitive manner**

We determined whether the direct binding between isorhamnetin and MEK1 or PI3-K occurred in an ATP-competitive manner. Results indicated that the amount of MEK1 bound to isorhamnetin-Sepharose beads remained constant with increasing concentrations of ATP (Fig. 6A) whereas the amount of PI3-K bound to isorhamnetin-Sepharose beads decreased with increasing concentrations of ATP (Fig. 6B). Thus, isorhamnetin bound to MEK1 in an ATP-noncompetitive manner and to PI3-K in an ATP-competitive manner. Thus, to predict the binding mode between isorhamnetin and MEK1 or PI3-K, we conducted a molecular modeling study. Considering the experimental observation that isorhamnetin binds to MEK1 noncompetitively with ATP, we docked this compound into the pocket separate from but adjacent to the ATP-binding site, similar to PD318088 in the crystal structure of the MEK1–PD318088 complex (Fig. 6C; ref. 24). The putative binding mode of isorhamnetin was also similar to that of PD318088. The hydroxyl groups at the 7-position can form a hydrogen bond with the backbone carbonyl group of Val127 in the ATP-noncompetitive binding site. In addition, isorhamnetin can form hydrophobic interactions with the side chains of Ile99, Ile141, Phe209, and Leu118. The methoxy group at the 3'-position and the hydroxyl group at the 4'-position can form hydrogen bonds with the backbone atoms of Ser212 in the activation loop of the nonphosphorylated MEK1 and the benzene ring moiety can form van der Waals interactions with Val211 and Leu215 in the activation loop. These interactions between isorhamnetin and the activation loop would render MEK1 catalytically inactive by stabilizing the inactive conformation of the activation loop.

To further investigate the binding mode of isorhamnetin to PI3-K, we also conducted a modeling study by using the crystal structure of PI3-K in complex with ATP or quercetin (25, 26). PI3-K consists of 4 domains: a Ras-binding domain, C2 domain, helical domain, and a catalytic domain. The catalytic domain of the enzyme consists of a C-lobe and N-lobe, with a fold similar to that of most protein kinases, and this structural similarity is also conserved in the ATP-binding site that is flanked by these 2 lobes. Consequently, ATP binds between these lobes in a manner similar to ATP binding in protein kinases. The N-
lobe and C-lobe are linked through a loop, called the "hinge region." The backbone of this loop interacts with the adenine moiety of ATP through hydrogen bonding. Considering the experimental result showing that isorhamnetin is an ATP-competitive inhibitor of PI3-K, we docked the compound to the ATP-binding site of PI3-K (Fig. 6D). The carbonyl group at the 4-position and the hydroxyl groups at the 5-position of isorhamnetin can form hydrogen bonds with the backbone atoms of Val887 in the hinge region of PI3-K. The hydroxyl groups at the 7- and 4-positions can also form hydrogen bonds with the side chains of Lys890 and Asp841, respectively. In addition, isorhamnetin would be sandwiched among the side chains of the hydrophobic residues in the ATP-binding site, including Met804, Trp812, Ile831, Ile879, and Ile881 from the N-lobe and Ala885, Phe961, Met953, and Ile963 from the C-lobe. The strong inhibitory activity of isorhamnetin for PI3-K may be attributable to this hydrogen bonding and hydrophobic interactions.

Discussion

EGFR is a RTK that mediates multiple signaling pathways including the MEK/ERKs and PI3-K pathways that regulate cell proliferation (27). It is an important drug target in cancer and acts as a strong prognostic factor (8). This receptor is expressed abundantly both in the basal layer of the epidermis and in the outer sheath of hair follicles and is highly overexpressed in NMSC (28). It mediates the signaling pathway induced by major etiologic factors of skin cancer including UV light and heavy metal exposure. Activated EGFR promotes AP-1 transactivation by mediating signaling pathways such as PI3-K and MEK/ERKs (7). These pathways induce many inflammatory genes, such as COX-2, which play pivotal roles in skin carcinogenesis (29). Here, we studied the inhibitory effects of a natural compound, isorhamnetin, on EGFR-mediated skin cancer.

To verify the inhibitory effects of isorhamnetin in EGFR-mediated skin cancer, we used 2 types of
Inhibition of Skin Cancer by Isorhamnetin

Figure 6. Isorhamnetin (IR) binds to MEK1 in an ATP-noncompetitive manner and to PI3-K in an ATP-competitive manner. Active MEK1 (A) or PI3-K (B): lane 1 (input control), MEK1 (A) and PI3-K (B) protein standard; lane 2 is the negative control, indicating that neither MEK1 (A) nor PI3-K (B) binds to Sepharose 4B; and lane 3 is the positive control, showing that MEK1 (A) or PI3-K (B) binds with isorhamnetin-Sepharose 4B. C and D, hypothetical model of MEK1 and PI3-K in complex with isorhamnetin. C, hypothetical model of the MEK1–isorhamnetin complex and its close-up view, isorhamnetin (atomic color) binds to the pocket adjacent to the ATP (orange)-binding site. PD318088 (white) is overlaid on the model structure of the MEK1–isorhamnetin complex for comparison. The partially disordered activation loop is colored yellow. The residues in gray ellipses are involved in hydrophobic interactions with isorhamnetin. The hydrogen bonds are depicted as white lines. D, hypothetical model of PI3-K in complex with isorhamnetin and its close-up view. The Ras-binding domain, C2 domain, and helical domain of PI3-K are colored in gray. The N-lobe, C-lobe, and hinge region of the catalytic domain are colored in yellow, purple, and cyan, respectively. Isorhamnetin (atomic color) binds to the ATP-binding site in the catalytic domain of PI3-K. The residues shown in gray ellipses represent the hydrophobic residues interacting with isorhamnetin. The hydrogen bonds are depicted as white lines.

experimental models, including an EGF-induced JB6 cell transformation assay and a mouse xenograft model of A431 cells. JB6 mouse epidermal cells are a well-developed cell culture system for studying tumor promotion ex vivo. This model comprises 2 phenotypes, promotion-sensitive (P+) and promotion-resistant (P−) JB6 mouse epidermal cell lines (30, 31). JB6 P+ cells, but not P− cells, are irreversibly transformed by a tumor promoter such as EGF. This process represents the preneoplastic to neoplastic progression. In contrast, the A431 cell line was derived from an 85-year-old woman with skin epidermal carcinoma. Because this cell line highly overexpresses EGFR, forms colonies when cultivated in soft agar, and develops tumors in nude mice, it is an excellent model for studying EGFR-mediated cellular signaling (32–34).
Isorhamnetin is a dietary flavonoid, found in apples, blackberries, and pears (35). It is also a major plasma metabolite of queretin, which is a highly abundant flavonoid found in many dietary plants (36). Recent studies showed that isorhamnetin exerts chemopreventive effects against cancer, cardiovascular disease, obesity, and Alzheimer’s disease (15, 17, 18). These studies have attributed the major chemopreventive mechanism of isorhamnetin to its antioxidant effects. Indeed, many reports have suggested that the health benefits of other flavonoids and isorhamnetin come from antioxidant effects because they are strong antioxidants (37). Nonetheless, the proposed antioxidant effects cannot explain all of the effects of most flavonoids, such as the low effective dose and specific inhibition of oncogenic signal transduction pathways. Our findings and other previous studies suggest that flavonoids can also act as specific kinase inhibitors (38).

A strong link has been reported between inflammation and carcinogenesis (39). COX-2 is a major mediator of inflammation, and its product, prostaglandin E₂, enhances carcinogenesis (22), increasing both the number of cells during the exponential growth phase and the number of colonies formed in soft agar (40). Knockout of Cox-2 results in reduced tumor formation and progression, and epidemiologic studies and animal experiments have shown that nonsteroidal anti-inflammatory drugs targeting COX-2 can reduce the incidence of colorectal carcinoma (41). Thus, COX-2 has been recognized as a molecular target for many chemopreventive and anti-inflammatory agents. In the present study, isorhamnetin inhibited EGF-induced COX-2 protein expression both in JB6 cells and in A431 cells. These results indicate that COX-2 is the target of isorhamnetin and that isorhamnetin has chemopreventive potential.

MEK1 is a dual-specificity protein kinase that phosphorylates ERK1/2 at specific tyrosine and threonine residues. MEK is activated by the phosphorylation of key serine residues in its catalytic domain by an upstream serine kinase, Raf. Many tumors express mutated Raf or MEK (42). A small-molecule inhibitor of MEK1 is capable of inhibiting up to 80% of the growth of human and murine colon carcinomas in mice (43). The inhibition of MEK1 attenuates neoplastic cell transformation (44). The expression of dominant-negative ERK2 shows a similar result (45, 46). p90RSK is also required for neoplastic cell transformation (47). PI3-K, a family of heterodimeric kinases, plays a pivotal role in the control of multiple cell signaling pathways leading to cell survival, motility, apoptosis, and proliferation. Several studies have shown that PI3-K/Akt pathway deregulation is associated with various types of cancer through subunit mutations or the regulation of apoptosis (26). PI3-K is also involved in neoplastic cell transformation and COX-2 expression (48). Thus, MEK1 and PI3-K are attractive targets for pharmacologic interventions in proliferative and inflammatory diseases.

In this study, isorhamnetin inhibited the phosphorylation of ERKs, p90RSK, Akt, and p70S6K. We suggest that the direct molecular targets of isorhamnetin are MEK1 and PI3-K. Isorhamnetin suppresses MEK1 kinase activity through direct binding, and because ERKs are substrates of MEK1, the inhibition of MEK1 by isorhamnetin leads to the inhibition of EGF-induced phosphorylation of ERKs. Subsequently, EGF-induced phosphorylation of p90RSK, which is the substrate of ERKs, is also inhibited by isorhamnetin. The inhibition of PI3-K activity by isorhamnetin might explain the manner in which isorhamnetin inhibits the phosphorylation of Akt and p70S6K.

In summary, isorhamnetin inhibits EGF-induced neoplastic transformation and COX-2 protein expression in JB6 cells and also reduces tumor growth and COX-2 expression in the A431 mouse xenograft model. This inhibition is mediated primarily through the blocking of the MEK/ERK/p90RSK and PI3-K/Akt/p70S6K signaling pathways and the subsequent suppression of AP-1 activity. Isorhamnetin strongly suppresses MEK1 and PI3-K activities. Overall, these results suggest that MEK1 and PI3-K are potent molecular targets for the suppression of neoplastic transformation by isorhamnetin.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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