Myricetin Suppresses UVB-Induced Skin Cancer by Targeting Fyn

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Abstract

Skin cancer is currently the most common type of human cancer in Americans. Myricetin, a naturally occurring phytochemical, has potent anticancer-promoting activity and contributes to the chemopreventive potential of several foods, including red wine. Here, we show that myricetin suppresses UVB-induced cyclooxygenase-2 (COX-2) expression in mouse skin epidermal JB6 P+ cells. The activation of activator protein-1 and nuclear factor-κB induced by UVB was dose-dependently inhibited by myricetin treatment. Western blot and kinase assay data revealed that myricetin inhibited Fyn kinase activity and subsequently attenuated UVB-induced phosphorylation of mitogen-activated protein kinases. Pull-down assays revealed that myricetin competitively bound with ATP to suppress Fyn kinase activity. Importantly, myricetin exerted similar inhibitory effects compared with 4-aminophenol, a well-known pharmacologic inhibitor of Fyn. In vivo mouse skin data also revealed that myricetin inhibited Fyn kinase activity directly and subsequently attenuated UVB-induced COX-2 expression. Mouse skin tumorigenesis data clearly showed that pretreatment with myricetin significantly suppressed UVB-induced skin tumor incidence in a dose-dependent manner. Docking data suggest that myricetin is easily docked to the ATP-binding site of Fyn, which is located between the N and C lobes of the kinase domain. Overall, these results indicated that myricetin exerts potent chemopreventive activity mainly by targeting Fyn in skin carcinogenesis. [Cancer Res 2008;68(14):6021–30]

Introduction

Skin cancer is currently the most common type of human cancer in Americans, and its incidence is increasing at an astonishing rate (1). Epidemiologic evidence and molecular studies suggest that nonmelanoma skin cancer is related to excessive exposure to the UV radiation in sunlight (2, 3). UV is divided into three subtypes, each of which has distinct biological effects: UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm; ref. 4). Although UVC is blocked by stratospheric ozone, UVB and UVA reach the surface of the earth and cause DNA damage, erythema, sunburn, immunosuppression, and, eventually, skin cancer (1). UVA, the major component of UV irradiation to which humans are exposed, is carcinogenic and causes photoaging and wrinkling of the skin. UVB is recognized as a complete carcinogen with relevance to human skin cancer (5, 6). Therefore, targeting UVB-induced signaling and signal molecules might be an effective strategy for preventing skin tumorigenesis.

Cyclooxygenase (COX), a rate-limiting enzyme for the oxidative conversion of arachidonic acids to prostaglandins, has two main isoforms: COX-1 and COX-2. COX-1 is a constitutively expressed isoform, and COX-2 is an inducible isoform (7). In human and murine skin cells, COX-2 is up-regulated in response to acute and chronic UVB irradiation (8). Celecoxib, a COX-2 inhibitor, has been shown to inhibit tumor formation in several animal models, including mouse skin (9). Several studies have found that up-regulated COX-2 expression in skin epidermis is involved in the development of skin cancer (10). Thus, developing an agent that can suppress COX-2 expression could be a critical strategy for the chemoprevention of skin cancer. The Src family kinases (SFK) consist of nine highly similar tyrosine kinases, and increased SFK activity is related to cellular events, including differentiation, proliferation, migration, and survival (11); SFKs are found in a variety of epithelial tumors, including ulcerative colitis, colonic adenocarcinoma, mammmary carcinoma, and murine cutaneous squamous cell carcinoma (12–15). Activation of Fyn, a nonreceptor tyrosine kinase and member of the Src family, plays a critical role in the development of skin cancer (16, 17). Interactions of Fyn with various signaling molecules regulate diverse biological functions, such as the progression of carcinogenesis (13, 18). Activated Fyn promotes oral cancer progression via Raf-extracellular signal-regulated kinase (ERK) and integrin βv signaling (19), and Fyn tyrosine kinase is a downstream mediator of Rho/PAK2 function in keratinocyte cell-cell adhesion (20). Multiple lines of evidence have shown a relationship between Fyn and skin abnormalities. Mice expressing double mutated fyn−/−fak−/− exerted abnormal thickness of the epidermis and hyperdifferentiation of keratinocytes (21). Fyn also mediated keratinocyte migration and squamous carcinoma invasion through the disruption of α6β4 integrin (22). Thus, Fyn may be a molecular target for chemoprevention of skin cancer.

In an increasing number of studies, many naturally occurring phytochemicals that are present in the human diet have been recommended as potential chemoprevention agents (23), but the search for novel natural agents and the determination of novel targets for chemoprevention is challenging. Myricetin (3,3',4',5,5',7-hexahydroxyflavone; Fig. 1A) is one of the major flavonoids found in various foods, including onions, berries, and grapes, as well as red wine (24–26). Research data have shown that myricetin exerts antioxidant, antitumor, and antiinflammatory effects (23, 27). A recent study showed that myricetin strongly inhibited tumor promoter–induced neoplastic cell transformation by direct inhibition of mitogen-activated protein kinase (MAPK)/ERK kinase.
(MEK) kinase activity (28). Moreover, its inhibitory effect was more potent than resveratrol, which is a well-known chemopreventive agent with anticancer effects (28). These accumulated data provide evidence that myricetin is a potent anticancer agent, but the underlying mechanism and target(s) in skin cancer are unclear. Here, we report that myricetin is an ATP-competitive inhibitor of Fyn and subsequently suppresses UVB-induced COX-2 expression in JB6 P+ mouse epidermal cells and in mouse dorsal skin. In a mouse skin tumorigenesis model, myricetin strongly suppressed UVB-induced incidence of mouse skin tumors.

Materials and Methods

Materials. Myricetin (>95% purity) and the antibody against β-actin were purchased from Sigma-Aldrich. Eagle’s MEM, gentamicin, and L-glutamine were obtained from Life Technologies-Bethesda Research Laboratories. Fetal bovine serum (FBS) was purchased from Gemini Bio-Products, and 4-amino-5-(4-chloro-phenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine was obtained from Calbiochem. The antibody against COX-2 was purchased from Cayman. Antibodies to detect phosphorylated p38 (Tyr180/Tyr182), total p38, phosphorylated c-Jun NH2 kinase (JNK; Thr185/Tyr185), total JNK, phosphorylated p90RSK (Thr359/Ser363), total p90RSK, phosphorylated MSK(Ser 376), total MSK, phosphorylated MEK1/2 (Ser217/221), total MEK, phosphorylated Raf (Ser259), and total Raf were purchased from Cell Signaling Biotechnology. Antibodies against phosphorylated Fyn (Thr12), phosphorylated ERK1/2 (Thr202/Tyr204), and total ERK were obtained from Santa Cruz Biotechnology. The active Fyn protein and antibody against Fyn were obtained from Upstate Biotechnology. CNBr-sepharose 4B, glutathione-sepharose 4B, [γ-32P]ATP, and the chemiluminescence detection kit were purchased from Amersham Pharmacia Biotech, and the protein assay kit was obtained from Bio-Rad Laboratories. G418 and the luciferase assay substrate were purchased from Promega. Eagle’s MEM was from Invitrogen.

Cell culture. The JB6 P+ mouse epidermal cell line was cultured in 5% FBS-MEM, 2 mmol/L L-glutamine, and 25 μg/mL gentamicin in monolayers at 37°C in a 5% CO2 incubator. The JB6 P+ mouse epidermal cell line was stably transfected with the activator protein-1 (AP-1), nuclear factor-κB (NF-κB), and COX-2 luciferase reporter plasmid and maintained in 5% FBS-MEM containing 200 μg/mL G418.

Animals. Female ICR mice and SKH-1 hairless mice (5 wk of age; mean body weight, 25 g) were purchased from the Institute of Laboratory Animal Resources at Seoul National University. Animals were acclimated for 1 wk before the study and had free access to food and water. The animals were
housed in climate-controlled quarters (24°C at 50% humidity) with a 12-h light/12-h dark cycle.

**Luciferase assay for AP-1, NF-κB, and COX-2 transactivation.** Confluent monolayers of JB6 P+ cells stably transfected with the AP-1, NF-κB, or COX-2 luciferase plasmid were harvested, and viable cells (8 × 10⁴) were incubated with the specific primary antibody at 4°C for 1 h before UVB irradiation. The cells were then washed twice with PBS and centrifuged for 10 min at 1,000 g. The cell lysate was incubated with protein-A/G beads (20 μg) for 3 h at 4°C. The mixture was processed, and radioactive incorporation was determined as described above for the ex vivo assay described above. Data are presented as the mean of data points from five mice in each group.

**Preparation of myricetin-sepharose 4B.** Myricetin-sepharose 4B freeze-dried powder (0.3 g) was suspended in 1 mmol/L HCl and the coupled solution [0.1 mol/L NaHCO₃ (pH 8.3) and 0.5 mol/L NaCl] was mixed and rotated at 4°C overnight. The medium was transferred to 0.1 mol/L Tris-HCl buffer (pH 8.0) and then rotated end over end at 4°C overnight. The medium was washed thrice with 0.1 mol/L acetate buffer (pH 4.0) containing 0.5 mol/L NaCl followed by a wash with 0.1 mol/L Tris-HCl (pH 8.0) containing 0.5 mol/L NaCl.

**Pull-down assays.** For the ex vivo pull-down assay, a cellular supernatant fraction of JB6 P+ cells (500 μg) was incubated with myricetin-sepharose 4B (or sepharose 4B alone as a control) beads (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/mL bovine serum albumin, 0.02 mmol/L phenylmethylsulfonyl fluoride (PMSF), and 1 μg protease inhibitor mixture]. After incubation with gentle rocking overnight at 4°C, the beads were washed five times with 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. For the in vivo pull-down assay, mice received topical application of 200 μL acetone alone or myricetin (5, 25, or 125 nmol) in 200 μL acetone on their shaved backs 1 h before UVB irradiation. Dorsal skin was prepared as described above for the in vivo Western blotting, and proteins were extracted as described above for Fyn immunoprecipitation and kinase assays. Then 50 μg of mouse skin extract was incubated with myricetin-sepharose 4B (or sepharose 4B alone as a control) beads (100 μL, 50% slurry) in reaction buffer as described for the ex vivo pull-down assay. Beads were incubated and washed, and proteins bound to the beads were analyzed by Western blot as described above.

**Mouse skin tumorigenesis analysis.** Skin carcinogenesis was induced using a UVB irradiation system in mice. The UVB radiation source (Bio-Link cross-linker: Vilber Lourmat) emitted at wavelengths of 254, 312, and 365 nm, with peak emission at 312 nm. SKH-1 mice were divided into four groups of 15 animals each. In the control mice, the dorsal skin was topically treated with 200 μL acetone only. In the UVB group of mice, the dorsal skin was topically treated with 200 μL acetone 1 h before UVB. The mice in the third and fourth groups received topical application of myricetin (8 or 20 nmol) in 200 μL acetone 1 h before UVB irradiation. The UVB dose was 0.18 J/cm² given thrice/week for 27 wk. The incidence of skin tumors was recorded weekly, and a tumor was defined as an outgrowth of >1 mm in diameter that persisted for 2 weeks or more. Tumor incidence, multiplicity, and volume were recorded every wk until the end of the experiment at the 27th week.

**Molecular modeling.** Insight II (Accelrys, Inc.) was used for the docking study and structure analysis with the crystal coordinates of the kinase domain of Fyn in a complex with staurosporine (accession code 2DQ7), which is available in the Protein Data Bank.⁶

**Statistical analysis.** As necessary, data were expressed as means ± SE, and the significant differences were determined using one-way ANOVA. A probability value of P < 0.05 was used as the criterion for statistical significance. All analyses were performed using Statistical Analysis Software (SAS, Inc.).

**Results**

Myricetin inhibits UVB-induced COX-2 expression and AP-1 and NF-κB activation. Previous studies have shown that abnormal induction of COX-2 plays an important role in
Multiple lines of evidence indicate that activation of AP-1 or NF-κB expression and mediate skin cancer (6, 10). Pretreatment of cells with myricetin significantly inhibited UVB-induced phosphorylation of p38 MAPK, JNKs, and ERKs in JB6 P+ cells. MAPKs, including p38 MAPK, JNKs, and ERKs mediate the up-regulation of AP-1 and NF-κB activity (6, 29, 30). Pretreatment of JB6 P+ cells with myricetin markedly inhibited UVB (0.05 J/cm²)-induced phosphorylation of p38 MAPK, JNKs, and ERKs (Fig. 2A) and also reduced UVB-induced phosphorylation of p90RSK and MSK (Fig. 2B), which are downstream of MAPKs. Recent studies have shown that phosphorylation of MAPKs and Src kinase is strongly related to the development of cancer (32). In particular, the Fyn/ERK pathway has been reported to regulate hyperkeratosis and skin cancer (21, 22). Therefore, we examined the effect of myricetin on the UVB-induced Fyn-RAF-MEK signaling pathway. UVB irradiation-induced up-regulation of RAF-MEK phosphorylation was significantly suppressed by myricetin. However, myricetin had no effect on UVB-induced phosphorylation of Fyn (Fig. 2C).

**Myricetin suppresses UVB-induced phosphorylation of p38 MAPK, JNKs, and ERKs in JB6 P+ cells.** MAPKs, including p38 MAPK, JNKs, and ERKs, play a crucial role in mediating UVB-induced skin cancer (6, 29–31). We first examined the effect of myricetin on UVB-induced phosphorylation of p38 MAPK, JNKs, and ERKs in JB6 P+ cells. The phosphorylation levels of p38, JNK, MEK, RAF, and Fyn were determined by Western blot analysis using specific antibodies against the corresponding phosphorylated and total proteins as described in Materials and Methods. Data are representative of three independent experiments that gave similar results. UV-induced skin cancer (6, 29–31). We first examined the effect of myricetin on UVB-induced phosphorylation of p38, JNK, and ERK in JB6 P+ cells stably transfected with a COX-2–luciferase plasmid. Myricetin inhibited UVB-induced phosphorylation of p38, JNK, p90RSK, MSK, MEK, RAF, and Fyn proteins were determined by Western blot analysis using specific antibodies against the corresponding phosphorylated and total proteins as described in Materials and Methods. Data are representative of three independent experiments that gave similar results.

**Myricetin inhibits Fyn kinase activity by competitively binding with ATP.** Because myricetin strongly blocked the UVB-induced phosphorylation of MAPKs, we hypothesized that, although myricetin did not affect UVB-induced phosphorylation of Fyn, it might still directly inhibit Fyn kinase activity. In vitro kinase assay data indicated that Fyn kinase activity was substantially induced by UVB (Fig. 3A) and was significantly inhibited in a dose-dependent manner by treatment with myricetin (Fig. 3B). The time response study showed that Fyn kinase activity in JB6 P+ cells peaked at 10 min after irradiation with UVB (0.05 J/cm²). The UVB condition [10 min and UVB (0.05 J/cm²)] was used in an additional study using cell lysates, which revealed that the 6-fold increase in Fyn kinase activity was considerably suppressed by pretreatment with myricetin ex vivo (Fig. 3C). Because myricetin inhibits Fyn kinase activity, we performed in vitro and ex vivo pull-down assays to determine whether myricetin interacts directly with Fyn. Results indicated that Fyn was bound with the myricetin-sepharose 4B beads (Fig. 3D, top, lane 3), but did not bind with the sepharose 4B beads alone (Fig. 3D, top, lane 2). We also observed ex vivo binding of myricetin and Fyn in JB6 P+ cell lysates (Fig. 3D, bottom), indicating that myricetin directly binds with Fyn. The binding ability of myricetin with Fyn was altered in a concentration-dependent manner in the presence of ATP (Fig. 3D, bottom, lanes 3–5), suggesting that myricetin competes with ATP for binding with Fyn. These results suggest that myricetin is an ATP-competitive inhibitor for suppressing Fyn kinase activity.

**Myricetin suppresses UVB-induced Fyn kinase activity, COX-2 expression, and phosphorylation of ERK, p38 MAPK, and JNK in vivo.** Many studies have shown that the rodent model is optimal for studying the mechanisms of skin carcinogenesis (30, 33, 34). We next investigated the effect of myricetin on UVB-induced Fyn kinase activity, COX-2 expression, and phosphorylation of MAPKs using mouse skin. Topical pretreatment with myricetin suppressed UVB-induced Fyn kinase activity in mouse dorsal skin extracts (Fig. 4A). We also found that Fyn from these extracts bound to myricetin-sepharose 4B beads (Fig. 4B, lane 3), but not to sepharose 4B beads alone (Fig. 4B, lane 2). These results suggest that myricetin significantly inhibited Fyn kinase activity by directly binding with Fyn kinase in mouse skin extracts. Further in vivo studies showed that topical treatment of myricetin significantly suppressed UVB-induced COX-2 protein expression (Fig. 4C) and markedly inhibited phosphorylation of ERKs, p38 MAPK, and NF-κB.
JNKs in mouse skin (Fig. 4D). These results clearly showed that UVB-induced Fyn kinase activity was significantly suppressed by topically applied myricetin and that its inhibitory effect is attributable to its regulation of Fyn kinase activity in mouse skin.

**Myricetin inhibits UVB-induced skin tumorigenesis in an SKH-1 hairless mouse model.** To further study the antitumorigenic activity of myricetin in vivo, we evaluated the effect of myricetin in the two-stage UVB-induced skin tumorigenesis model (33, 34). Photographic data show that myricetin inhibited UVB-induced mouse skin cancer development compared with the UVB-only irradiated mice (Fig. 5A). Topically applied myricetin (8 or 20 nmol) on mouse skin resulted in a significant inhibition of tumor incidence of 67.4% and 81.7%, respectively (\( P < 0.001 \) versus the UVB irradiation group, \( n = 15 \); Fig. 5B). The volume of tumors developed in UVB-treated mouse skin was significantly attenuated by myricetin treatment (Fig. 5C). Furthermore, the levels of COX-2 expression in the myricetin-treated UVB groups were much lower than COX-2 levels in the UVB-only group (Fig. 5D). Overall, these results indicate that myricetin might serve as an effective chemopreventive agent against UVB-mediated skin cancer.

**Discussion**

A previous study showed that myricetin is a potent inhibitor of bay-region diol-epoxides of polycyclic aromatic hydrocarbon adduct formation in the epidermis and lung of SENCAR mice (35). Myricetin was reported to reduce tumor formation using 7,12-dimethylbenz(a)anthracene, benzo(a)pyrene, or N-methyl-N-nitrosoureas initiation and promotion in a skin tumorigenesis model (36). We have shown previously that myricetin strongly...
inhibits 12-O-tetradecanoylphorbol-13-acetate or epidermal growth factor (EGF)-induced cell transformation by directly inhibiting MEK activity (28). In the present study, we showed the chemopreventive effect of myricetin on UVB-induced skin cancer and identified molecular mechanism(s) and target(s). COX-2 is induced by cytokines and tumor promoters, and aberrant COX-2 expression has been detected in many human malignancies, including skin cancer. Selective COX-2 inhibitors are known to possess protective effects against UVB-mediated skin cancer. AP-1 and NF-κB are transcription factors that are extremely important in biological events, such as cell proliferation, inflammation, metastasis, and cell transformation (30, 31). Many studies have shown that AP-1 and NF-κB are activated by various tumor promoters and are ideal targets for chemopreventive agents in various cancers, including skin cancer (30). An in vivo mouse skin model indicated that AP-1 and NF-κB transactivation plays a critical role in tumor promotion (37). The promoter region of the Cox-2 gene has binding sites for various transcription factors, including AP-1 and NF-κB (6, 38). We performed a luciferase assay to detect Cox-2 promoter activity and AP-1 or NF-κB transcription activity. Our results showed that myricetin inhibits COX-2 expression by regulating Cox-2 promoter activity, which was associated with the inhibition of AP-1 and NF-κB transcription activation. Together, these results suggested that inhibition of UVB-induced COX-2 expression by myricetin

Figure 4. In vivo study for the effects of myricetin on UVB-induced Fyn kinase activity, COX-2 expression, and phosphorylation of ERK, p38 MAPK, and JNK in mouse dorsal skin. A, myricetin inhibits UVB-induced Fyn kinase activity in mouse skin extracts. For the Fyn kinase assay, dorsal skin protein lysates were prepared from the epidermis, and the assays were carried out as described in Materials and Methods. Each band was quantified by densitometry. Columns, mean (n = 5); bars, SE. #, significant difference (P < 0.05) between the control group and the group exposed to UVB (0.5 J/cm²) alone; *, significant difference (P < 0.05) between groups treated with UVB and myricetin and the group exposed to UVB alone. B, myricetin specifically binds with Fyn in mouse skin extracts. The Fyn-myricetin binding in vivo was confirmed by immunoblotting using an antibody against Fyn. lane 1 (input control), mouse dorsal skin lysate; lane 2 (control), a lysate of mouse dorsal skin precipitated with sepharose 4B beads as described in Materials and Methods; lane 3, mouse dorsal skin lysate precipitated by myricetin-sepharose 4B affinity beads. Each experiment was performed thrice. C, myricetin significantly inhibits UVB-induced COX-2 expression in mouse skin extracts. The levels of COX-2 and β-actin were determined by Western blot analysis using specific antibodies against the corresponding COX-2 and β-actin. Each band was quantified by densitometry. Columns, mean (n = 5); bars, SE. ##, significant difference (P < 0.01) between the control group and the group exposed to UVB alone; * and **, significant difference at P < 0.05 and P < 0.05, respectively, between groups treated with myricetin and irradiated with UVB and the group exposed to UVB alone. D, myricetin inhibits phosphorylation of ERK, p38 MAPK, and JNK in mouse skin extracts. The levels of phosphorylated and total ERK, p38 MAPK, and JNK were determined by Western blot analysis using specific antibodies against the corresponding phosphorylated and total proteins. Data are representative of three independent experiments with similar results (n = 5).
might be at least partially due to an inhibition of AP-1 and NF-κB transactivation.

Previous studies indicated that MAPKs are key molecules activated in response to UVB irradiation and play a critical role in the transcriptional activation of AP-1 and NF-κB (31, 38). Therefore, we determined whether the suppression of MAPK phosphorylation by myricetin could attenuate UVB-stimulated COX-2 expression through the inhibition of AP-1 or NF-κB transcription activity. These results showed that myricetin inhibited UVB-induced phosphorylation of ERK, p38 MAPK, JNK, p90RSK, and MSK. Because myricetin suppressed the activation of MAPK signaling, we hypothesized that the molecular target of myricetin to inhibit UVB-induced COX-2 expression might be an upstream kinase of MAPKs. Increased SFK activity is related to many human cancers, including ulcerative colitis, colonic adenocarcinoma, mammary carcinoma, and murine cutaneous squamous cell carcinoma (12–15). A recent report revealed that Fyn regulates UVB-induced phosphorylation of histone H3 and MAPK in JB6 P+ and HaCaT human keratinocytes (39). Based on the above studies and data, we hypothesized that Fyn kinase activity would play a key role in UVB-induced COX-2 expression by regulating MAPKs. Our results clearly showed that myricetin significantly inhibited Fyn kinase activity and skin cancer.

**Figure 5.** The effect of myricetin on UVB-induced skin carcinogenesis in the SKH-1 hairless mouse. A, external appearance of tumors. B, myricetin strongly inhibits UVB-induced cancer incidence in the SKH-1 hairless mouse. The control mice (n = 15) received a topical treatment of 200 μL acetone alone (no UVB) and the experimental mice (n = 15) were topically treated with 200 μL acetone before UVB (0.18 J/cm²) exposure 3 d/wk for 27 wk. The mice in the third and fourth groups received topical application of myricetin (8 or 20 nmol per mouse in 200 μL acetone) on the dorsal surface 1 h before UVB (0.18 J/cm²) irradiation 3 d/wk for 27 wk. The incidence of skin tumors was recorded weekly, and a tumor was considered to occur when an outgrowth of >1 mm in diameter persisted for 2 wk or longer. Tumor incidence and multiplicity were recorded every week until the end of the experiment at 27 wk. C, myricetin strongly suppresses UVB-induced tumor volume in the SKH-1 hairless mouse. The mice were treated as for B. At the end of study, the dimension of all tumors on each mouse was recorded. Tumor volumes were calculated using the hemiellipsoid model formula: tumor volume = 1 / 2 (4π / 3) (l / 2) (w / 2) h, wherein l is length, w is width, and h is height. D, myricetin strongly inhibits UVB-induced COX-2 expression in the SKH-1 hairless mouse. Skin samples from mice were analyzed for COX-2 expression by immunoblotting. Quantification of COX-2 immunoblot results was normalized to β-actin followed by statistical analysis of relative image density. ##, significant difference (P < 0.01) between the control group and the group exposed to UVB alone; **, significant difference at P < 0.05 between groups treated with myricetin and irradiated with UVB and the group exposed to UVB alone.
inhibited Fyn kinase activity and that the inhibition resulted from ATP-competitive binding of myricetin with Fyn. Furthermore, PP2, a well-known pharmacologic inhibitor of Fyn, and small interfering RNA against Fyn inhibited COX-2 expression by blocking phosphorylation of MAPKs. These results indicated that myricetin suppressed UVB-induced COX-2 expression and subsequently attenuated UVB-induced phosphorylation of MAPKs by regulating Fyn kinase activity.

We further showed that myricetin inhibited UVB-induced COX-2 expression in mouse skin. An in vivo study also confirmed previous results showing that myricetin suppressed UVB-induced Fyn kinase activity and subsequently attenuated phosphorylation of MAPKs in mouse skin extracts. We found that the inhibitory effect of myricetin on Fyn kinase activity induced the suppression of MAPKs phosphorylation and most likely was due to myricetin specifically binding with Fyn in JB6 P+ cells and mouse skin. In melanoma progression, activation of Fyn leads to melanocyte differentiation by modified MAPK activity (40). Increased epidermal Fyn levels are associated with the activation of ERK, signal transducers and activators of transcription-3 (STAT-3), and PDK-1 in keratin 14-Fyn (K14) transgenic mice (41). A recent study showed that binding between EGCG and Fyn reduced EGF-induced cell transformation and p38 phosphorylation in JB6 P+ cells (42). Previous studies also suggest a relationship between Fyn and various transcription factors, including activating transcription factor-2 (42), STAT (43, 44), and cAMP-responsive element binding protein (38). These results suggest that myricetin inhibits UVB-induced skin carcinogenesis through the inhibition of transcription factors and MAPKs by targeting Fyn.

A modeling study was performed using the crystal structure of the Fyn kinase domain in complex with staurosporine, a well-known kinase inhibitor (45). Myricetin easily docked to the ATP-binding site of Fyn, which is located between the N and C lobes of the kinase domain (Fig. 6A and B). Myricetin can form hydrogen bonds with the backbone of the hinge region of Fyn, as seen in other ATP-competitive kinase inhibitors. The docking data indicate that myricetin was easily docked to the ATP-binding site of Fyn. Our pull-down assay, which shows that myricetin competes with ATP for binding with Fyn, supports the docking results. The hydroxyl group at the 4β position works as a hydrogen bonding acceptor in the interaction with the backbone amide group of Met344, and the hydroxyl group at the 3β position is a hydrogen bonding donor in the interaction with the backbone carbonyl group of Glu342. At the same time, it can also work as a hydrogen bonding acceptor to interact with the side chain of Thr341. The hydroxyl group at the 7 position would make a hydrogen bond with the side chain of Glu113 and the hydroxyl group at the 5 position could also form a hydrogen bond with the backbone carbonyl group of Asn278. In addition, the inhibitor would be sandwiched by hydrophobic residues, including Leu276, Val284, and Ala296 from the N-lobe and Val326, Leu396, and Ala406 from the C-lobe, and thereby lead to the high activity of the inhibitor for Fyn. Further studies with X-ray crystallography to determine the inhibitor complex structure would elucidate the exact binding mode of myricetin and Fyn.

7 Unpublished observation.
Clinical observations and epidemiologic data strongly suggest that nonmelanoma skin cancer is related to chronic exposure to UV (4, 6, 30). Our results clearly showed that topical application of myricetin markedly inhibits the formation of skin cancer in the SKH-1 hairless mouse model. This inhibition occurs mainly through the blocking of Fyn, suggesting that Fyn is a critical target for myricetin in mediating COX-2 expression through the inhibition of MAPKs via regulation of AP-1 and NF-κB activity. A simplified depiction of our proposed antiskincancer mechanism of myricetin is shown in Fig. 6C. Therapeutic inhibition of Fyn kinase activity by myricetin might provide clinical benefit in skin cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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