Inhibitory Effects of Furanocoumarins From the Roots of *Angelica dahurica* on Ionizing Radiation-Induced Migration of A549 Human Non-Small Cell Lung Cancer Cells

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Abstract
Radiation therapy is a very effective tool for the treatment of advanced human lung cancers. However, as one of its malignancy-promoting behaviors, ionizing radiation (IR) increases cell migration and radiation resistance in several lung cancer cells, including non-small cell lung cancer (NSCLC) cells. As part of our ongoing search for potent radiotherapy enhancers from medicinal herbs, a chloroform-soluble fraction of the roots of *Angelica dahurica* was subjected to phytochemical investigation, leading to the isolation of 8 furanocoumarins. Of these, psoralen (1), xanthotoxin (2), and bergapten (3) inhibited IR-induced migration at a non-cytotoxic concentration (50 μM) in human NSCLC A549 cells. This study is the first to report on the inhibitory activities of these constituents of *A. dahurica* against IR-induced cancer metastasis.

Keywords
*Angelica dahurica*, apiaceae, furanocoumarin, radiation-induced migration, non-small-cell lung cancer cells

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Lung cancer is one of the most common types of cancer and is the leading cause of cancer deaths worldwide.¹ In recent years, the annual incidence of lung cancer has been about 1.8 million people, and the annual death rate, about 1.6 million people worldwide, accounting for nearly 20% of all cancer deaths.²,³ Non-small cell lung cancer (NSCLC) accounts for about 85% of all lung cancers, and many patients are diagnosed at an advanced stage.³ Most NSCLC patients are diagnosed with locally advanced or metastatic diseases, and the prognosis for these patients is very inadequate.⁴ Treatment of lung cancer includes surgical resection, chemotherapy, and radiotherapy, and among them, surgical resection is the preferred treatment but is limited to early stages (stages I and II).⁵ Radiotherapy and chemotherapy are considered the standard therapy for inoperable stage lung cancer (stages III and IV).⁵ However, in some cases, radiotherapy promotes malignant behaviors, such as local recurrence or distal metastasis. These consequences may cause the regrowth or spread of cancer cells that have survived radiation therapy. In vitro studies have shown that sublethal doses of ionizing radiation (IR) increase the migration and invasion of various cancer cell lines, including lung, glioma, hepatocellular carcinoma, and pancreatic cancer cells,⁶ and *in vivo* studies have suggested that radiotherapy of primary tumor sites may promote metastasis.⁷ Therefore, it is necessary to develop radiosensitizers that can inhibit malignant behavior caused by radiotherapy and simultaneously enhance its effectiveness.

We have previously reported that sublethal doses of IR increase sulfatase 2 (SULF2) expression via the p53 transcription factor, which mediates the migration and invasion of...
cancer cells. This appears to occur through mechanisms that stimulate the β-catenin, interleukin-6, signal transducer and activator of transcription 3, and Bel-4 signaling pathway or the phosphoinositide 3-kinase, Akt, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway. Moreover, it is reported that increased SULF2 from sublethal doses of IR regulates IR-induced cancer cell invasion via mitochondrial superoxide dismutase 2 (SOD2). These results suggest that IR promotes cancer cell invasion by activating different signaling pathway mediators, such as Bel-4, NF-κB, and SOD2.

Angelica Dahuricae Radix, the dried root of Angelica dahurica (Fisch. ex Hoffm.) Benth. et Hook. (Apiaceae) possesses medicinal properties and has been used as a traditional Chinese medicine for treating stomach ache, headache, toothache, abscesses, nose congestion, and dysmenorrhea. In several published studies, compounds 1-3 have shown potent anticancer activity in numerous cancer cell lines but these have not included lung cancer. For example, psoralen (1) inhibited proliferation by modulating the Wnt/β-catenin pathway in breast cancer cells. Xanthotoxin (2) has been reported to possess antitumor activity in breast cancer cells and inhibits the activation of AP-1 and NF-κB subunits. NF-κB has been implicated in numerous age-related diseases, including cancer. Finally, bergapten (3) decreased cell proliferation of breast cancer cells by inducing apoptosis via p53 signaling. Although the cancer-inhibiting properties of these compounds were documented, to the best of our knowledge, this is the first report on their biological activities relating specifically to cancer metastasis. Therefore, we suggest that compounds 1-3 may be effective in improving the therapeutic effects of radiotherapy. However, further study on the antivasive effects and the mechanisms of these compounds is required in order for them to become useful as radiosensitizers and enhancers during radiotherapy.

**Experimental**

**General**

Optical rotations were measured on a JASCO P-2000 polarimeter. One-dimensional (1D) and 2D NMR spectra were recorded on a UNITY INOVA 400 MHz FT-NMR instrument with tetramethylsilane as an internal standard. Mass spectrometry (MS) was performed on an Agilent 6550 ifunnel liquid chromatography/MS-quadrupole time-of-flight system. Silica gel (230-400 mesh, Merck, Germany), RP-C18 (YMC gel ODS-A, 12 nm, S-150 µm, YMC Co., Japan), and Sephadex LH-20 (GE Healthcare Bio-Science AB, Uppsala, Sweden) were used for column chromatography (CC). Thin-layer chromatographic analysis was performed on Kieselgel 60 F254 (silica gel, 0.25 mm layer thickness, Merck, Germany) and RP-18 F254s (Merck, Germany).
Figure 2. Effects of furanocoumarins 1-8 on ionizing radiation (IR)-induced A549 cell migration (a–h). (left panels) Wound healing assay was performed to examine the effects of furanocoumarins (50 µM) on the IR-induced migration of A549 cells. (right panels). Relative wound width was calculated as the ratio of the remaining wound width at the given time point to the original width at 0 hours. Data represent the mean ± standard deviation (n = 3); #P < 0.05; ##P < 0.005; *P > 0.05 vs the control.
Plant Materials

Angelicae Dahuricae Radix (the roots of *A. dahurica* (Fisch. ex Hoffm.) Benth. et Hook. were purchased from the Nonglim Oriental Herbal market in Seoul, South Korea, in July 2014 and identified by Professor Je-Hyun Lee (College of Oriental Medicine, Dongguk University). A voucher specimen (no. EA347) has been deposited at the College of Pharmacy, Ewha Woman’s University.

Extraction and Isolation

The roots of *A. dahurica* (4.8 kg) were extracted with CHCl₃ (3 × 6 L) overnight at room temperature. The CHCl₃ extract (115 g) was separated by silica gel column chromatography (CC) using CHCl₃–acetone (1:0 to 0:1, v/v), affording 7 fractions (F₁–F₇). Fraction F₂ (43 g) was subjected to silica gel CC using hexanes–EtOAc (9:1 to 1:1, v/v) to obtain subfractions F₀₂₀₁–F₀₂₁₉. Compound 6 (3.3 g, 0.69% w/w) was precipitated from F₀₂₁₉ with EtOAc. Fraction F₀₂₁₄ (6 g) was subjected to RP-C₁₈ CC with acetonitrile (MeCN)–water (H₂O) (1:1, v/v) as a solvent system to yield 1 (325.8 mg, 0.0068% w/w), 3 (9.5 mg, 0.0002% w/w), 4 (659.5 mg, 0.014% w/w), and 5 (761.5 mg, 0.016% w/w). Fraction F₀₂₁₅ (0.1 g) was subjected to RP-C₁₈ CC with MeCN–H₂O (1:1, v/v), to furnish 2 (0.9 mg, 0.000019% w/w). Fraction F₀₂₁₂ (0.3 g) was subjected to RP-C₁₈ CC with MeCN–H₂O (1:1, v/v), to furnish 2 (0.9 mg, 0.000019% w/w). Fraction F₀₂₁₂ (0.3 g) was chromatographed over ODS-A using gradient mixtures of MeCN–H₂O (1:1 to 2:1, v/v) to yield 7 (9.1 mg, 0.00019% w/w). Compound 8 (497.5 mg, 0.01% w/w) was precipitated from F₄ in acetone.

Cell Culture

NSCLC A549 cells were obtained from the American Type Culture Collection (Rockville, MD, USA). Cells were cultured in RPMI-1640 medium (Hyclone, Logan, UT, USA) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone) at 37°C in a humidified atmosphere with 5% carbon dioxide.

Wound Healing Assay

Wound healing assays were performed as described previously. Briefly, A549 cells were seeded in a 24-well plate (3.5 × 10⁵ cells/well) containing plastic inserts (Cell Biolabs Inc., San Diego, CA, USA) for the generation of a wound field and incubated for 24 hours. After removing the inserts from the wells, cells were exposed to 10 Gy of γ-irradiation using a ¹³⁷Cs γ-ray source (Atomic Energy of Canada, Mississauga, Canada) at a dose rate of 3 Gy/min. The irradiated cells were incubated with 50 µM of each compound. Images were taken at time points of 0, 24, and 48 hours and analyzed for cell migration using an AE31 microscope (Motic, Hong Kong).

Cell Viability Assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used for determining the cytotoxicity of compounds in A549 cells. The cells (2 × 10³ cells/well) were seeded in 96-well plates and incubated for 24 hours and treated with various concentrations of each compound (0, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100, and 200 µM) for 24 hours. Subsequently, 50 µL of MTT solution (2 mg/mL) was added to each well and incubated for 3 hours. Formazan crystals generated in living cells were dissolved in 200 µL/well of dimethyl sulfoxide, and the absorbance of individual wells was measured at 570 nm using the Gemini XPS Microplate Reader (Molecular Devices, Sunnyvale, CA, USA).

Statistical Analysis

All experiments were replicated at least 3 times. Statistical significance was determined using Student’s *t*-test or one-way analysis of variance using GraphPad software (LaJolla, CA, USA). The 50% inhibitory concentration was calculated from a...
concentration–response analysis performed using GraphPad software (LaJolla, CA, USA).

**Declaration of Conflicting Interests**

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**References**

1. Siegel RL, Miller KD, Jemal A. Cancer statistics. *CA Cancer J Clin*. 2019;69(1):7-34. doi:10.3322/caac.21551

2. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics. *CA Cancer J Clin*. 2015;65(2):87-108. doi:10.3322/caac.21262

3. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2015;136(5):E359-E386. doi:10.1002/ijc.29210

4. Zappa C, Mousa SA. Non-small cell lung cancer: current treatment. *Biochim Biophys Acta*. 2015;1856(2):189-210. doi:10.1016/j.bbcan.2015.08.002

5. Lemjabbar-Alaoui H, Hassan OU, Yang Y-W, et al. Lung cancer: biology and treatment options. *Biochem Biophys Acta*. 2015;1856(2):189-210. doi:10.1016/j.bbabio.2015.08.002

6. Ho J-N, Kang KY, Lee S-S, et al. Bel-XI and STAT3 mediate malignant actions of gamma-irradiation in lung cancer cells. *Cancer Sci*. 2010;101(6):1417-1423. doi:10.1111/j.1349-7006.2010.01552.x

7. Park C-M, Park M-J, Kwak H-J, et al. Ionizing radiation enhances matrix metalloproteinase-2 secretion and invasion of glioma cells through Sre/epidermal growth factor receptor-mediated p38/Akt and phosphatidylinositol 3-kinase/Akt signaling pathways. *Cancer Res*. 2006;66(17):8511-8519. doi:10.1158/0008-5472.CAN-05-4340

8. Cheng JC, Chou CH, Kuo ML, et al. Radiation-enhanced hepatocellular carcinoma cell invasion with MMP-9 expression through PI3K/Akt/NF-kappaB signal transduction pathway. *OncoGene*. 2006;25(53):7009-7018. doi:10.1080/13578800600924292

9. Li D, Qu C, Ning Z, et al. Radiation promotes epithelial-to-mesenchymal transition and invasion of pancreatic cancer cell by activating carcinoma-associated fibroblasts. *Am J Cancer Res*. 2016;6(10):2192-2206.

10. Camphausen K, Moses MA, Beecken WD, et al. Radiation therapy to a primary tumor accelerates metastatic growth in mice. *Cancer Res*. 2001;61(5):2207-2211.

11. Jung C-H, Ho J-N, Park JK, et al. Involvement of Suhf2 in γ-irradiation-induced invasion and resistance of cancer cells by inducing IL-6 expression. *Oncotarget*. 2016;7(13):16090-16103. doi:10.18632/oncotarget.7449

12. Jung C-H, Han A-R, Chung H-J, et al. Linarin inhibits radiation-induced cancer invasion by downregulating MMP-9 expression via the suppression of NF-κB activation in human non-small-cell lung cancer A549. *Nat Prod Res*. 2019;33(24):3582-3586. doi:10.1080/14787484.2018.1484460

13. Jung C-H, Kim EM, Song J-Y, et al. Mitochondrial superoxide dismutase 2 mediates γ-irradiation-induced cancer cell invasion. *Exp Mol Med*. 2019;51(2):1-10. doi:10.1038/s12276-019-0207-5

14. Kim CM, Shin MK, Ahn DK, et al. Dictionary of chinese material medical. 4. Seoul: Jungdam Publishing; 1997.

15. Li B, Zhang X, Wang J, et al. Simultaneous characterisation of fifty coumarins from the roots of Angelica dahurica by off-line two-dimensional high-performance liquid chromatography coupled with electrospray ionisation tandem mass spectrometry. *Phytochem Anal*. 2014;25(3):229-240. doi:10.1002/pca.2496

16. Piao XL, Park IH, Baek SH, et al. Antioxidative activity of furanocoumarins isolated from angelicae dahuriae. *J Ethnopharmacol*. 2004;93(2-3):243-246. doi:10.1016/j.jep.2004.03.054

17. Yang W-Q, Song Y-I, Zhu Z-X, et al. Anti-inflammatory dimeric furanocoumarins from the roots of Angelica dahurica. *Fitoterapia*. 2015;105:187-193. doi:10.1016/j.fitote.2015.07.006

18. Wang KS, Lv Y, Wang Z, et al. Imperatorin efficiently blocks TNF-α-mediated activation of ROS/PI3K/Akt/NF-κB pathway. *Oncol Rep*. 2017;37(6):3397-3404. doi:10.3892/or.2017.5581

19. Li D, Wu L. Coumarins from the roots of Angelica dahurica cause anti-allergic inflammation. *Exp Ther Med*. 2017;14(1):874-880. doi:10.3892/etm.2017.4569

20. Mi C, Ma J, Wang KS, et al. Imperatorin suppresses proliferation and angiogenesis of human colon cancer cell by targeting HIF-1α via the mTOR/p70S6K/mTOR/p70S6K/4E-BP1 and MAPK pathways. *J Ethnopharmacol*. 2017;203:27-38. doi:10.1016/j.jep.2017.03.033

21. Kang TJ, Lee SY, Singh RP, et al. Anti-Tumor activity of oxypeucedanin from Ostericum koreanum against human prostate carcinoma DU145 cells. *Acta Oncol*. 2009;48(6):895-900. doi:10.1080/028418609028242925

22. Marumoto S, Miyazawa M. β-Secretase inhibitory effects of furanocoumarins from the root of Angelica dahurica. *Phytomther Res*. 2010;24(4):510-513. doi:10.1002/ptr.2967

23. Masuda T, Takasugi M, Anetai M. Psoralen and other linear furanocoumarins with affinity to brain benzodiazepine receptors. *Phytochemistry*. 1998;47(1):13-16. doi:10.1016/S0031-9422(97)00528-1

24. Bergendorff O, Dekerniondjan K, Nielsen M, et al. Furanocoumarins with affinity to brain benzodiazepine receptors in vitro. *Phytochemistry*. 1997;44(6):1121-1124. doi:10.1016/S0031-9422(96)00703-0

25. Baek NI, Ahn EM, Kim HY, et al. Furanocoumarins from the root of Angelica dahurica. *Arq Pharm Res*. 2000;23(5):467-470. doi:10.1007/BF02976574

26. Youkwan J, Suthivaiyakit S, Sutthivaiyakit P. Citrusosides A-D and furanocoumarins with cholinesterase inhibitory activity from the fruit peels of Citrus hystrix. *J Nat Prod*. 2010;73(11):1879-1883. doi:10.1021/np100531x
27. Nielsen BE, Lemmich J, Lemmich J. Constituents of umbelliferous plants. IX. The configuration of (+)-oxypeucedanin hydrate and related coumarins. Acta Chem Scand. 1969;23(3):962-966. doi:10.3891/acta.chem.scand.23-0962

28. Wang X, Xu C, Hua Y, et al. Psoralen induced cell cycle arrest by modulating Wnt/β-catenin pathway in breast cancer cells. Sci Rep. 2018;8(1):14001. doi:10.1038/s41598-018-32438-7

29. Abdel Hafez OM, Amin KM, Abdel-Latif NA, et al. Synthesis and antitumor activity of some new xanthotoxin derivatives. Eur J Med Chem. 2009;44(7):2967-2974. doi:10.1016/j.ejmech.2009.01.006

30. Lee S-B, Lee WS, Shin J-S, et al. Xanthotoxin suppresses LPS-induced expression of iNOS, COX-2, TNF-α, and IL-6 via AP-1, NF-xB, and JAK-STAT inactivation in RAW 264.7 macrophages. Int Immunopharmacol. 2017;49:21-29. doi:10.1016/j.intimp.2017.05.021

31. Tak PP, Firestein GS. NF-κB: a key role in inflammatory diseases. J Clin Invest. 2001;107(1):7-11. doi:10.1172/JCI11830

32. Pattanayak SP, Bose P, Sunita P, et al. Bergapten inhibits liver carcinogenesis by modulating LXR/PI3K/Akt and IDOL/LDLR pathways. Biomed Pharmacother. 2018;108:297-308. doi:10.1016/j.biopha.2018.08.145

33. Santoro M, Guido C, De Amicis F, et al. Bergapten induces metabolic reprogramming in breast cancer cells. Oncol Rep. 2016;35(1):568-576. doi:10.3892/or.2015.4327