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Research Article

Identification of Arctigenin as an Antitumor Agent Having the Ability to Eliminate the Tolerance of Cancer Cells to Nutrient Starvation

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Abstract

Tumor cells generally proliferate rapidly and the demand for essential nutrients as well as oxygen always exceeds the supply due to the unregulated growth and the insufficient and inappropriate vascular supply. However, cancer cells show an inherent ability to tolerate extreme conditions, such as that characterized by low nutrient and oxygen supply, by modulating their energy metabolism. Thus, targeting nutrient-deprived cancer cells may be a novel strategy in anticancer drug development. Based on that, we established a novel screening method to discover anticancer agents that preferentially inhibit cancer cell viability under the nutrient-deprived condition. After screening 500 medicinal plant extracts used in Japanese Kampo medicine, we found that a CH_2Cl_2-soluble extract of *Arctium lappa* exhibited 100% preferential cytotoxicity under the nutrient-deprived condition at a concentration of 50 μg/mL with virtually no cytotoxicity under nutrient-rich condition. Further bioassay-guided fractionation and isolation led to the isolation of arctigenin as the primary compound responsible for such preferential cytotoxicity; the compound exhibited 100% preferential cytotoxicity against nutrient-deprived cells at a concentration of 0.01 μg/mL. Furthermore, arctigenin was also found to strongly suppress the PANC-1 tumor growth in nude mice, as well as the growth of several of the tested pancreatic cancer cell lines, suggesting the feasibility of this novel antiausterity approach in cancer therapy. Further investigation of the mechanism of action of arctigenin revealed that the compound blocked the activation of Akt induced by glucose starvation, which is a key process in the tolerance exhibited by cancer cells to glucose starvation. (Cancer Res 2006; 66(3): 1751-7)

Introduction

A wealth of accumulated clinical experience and indigenous knowledge of traditional medicine is still waiting to be tapped as an important source for the modern drug discovery process. In traditional medicine systems, medicinal plants and their extracts have formed the main basis for the treatment of diseases for thousands of years. The unique and very broad chemical diversity of medicinal plant extracts constitutes immense potential in the search for novel medicaments.

Pancreatic cancer is one of the most serious forms of cancer that shows resistance to almost all known chemotherapeutic agents because of resistance of the cells to apoptosis (1). Almost all patients of pancreatic cancer rapidly develop metastases and die within a short period of time after the diagnosis; this cancer is associated with the lowest 5-year survival rates known for cancers (2, 3) and continues to be one of the major health problems that remain unresolved at the start of the 21st century. Currently, surgery is the only treatment modality that offers any prospect of potential cure. 5-Fluorouracil and gemcitabine are the most commonly used chemotherapeutic agents for palliative therapy of advanced pancreatic cancer. However, recent advances in the molecular biology of pancreatic cancer and the identification of molecular signals that mediate the resistance of the cancer cells to apoptosis have prompted the development application of a novel screening strategy for drugs with activity against this cancer from natural sources (4).

Earlier, we reported that certain pancreatic cancer cell lines, such as PANC-1, AsPC-1, BxPC-1, and KP-3, exhibit marked tolerance and survive for prolonged periods of time even under extremely nutrient-starved conditions, and that a high expression level of protein kinase B (PKB)/Akt was associated with this tolerance of the cells to nutrient starvation (5). Tumor cells, in general, are well known to show high glycolytic activity and, as the tumor progresses through the multiple steps of carcinogenesis and increases in size, it is faced with an insufficient supply of oxygen and other nutrients, including glucose, and growth factors. Thus, development of drugs aimed at countering this resistance of the cells to nutrient deprivation may serve as a novel biochemical approach to cancer therapy (5–7).

In this regard, we recently screened 500 medicinal plants used in Kampo medicine for their preferential cytotoxicity against PANC-1 cancer cell lines under nutrient-deprived conditions and found that a dichloromethane (CH_2Cl_2)-soluble extract of *Arctium lappa* exhibited 100% preferential cytotoxicity against nutrient-deprived cells at the concentration 50 μg/mL. Further activity-guided fractionation and separation led to the isolation of arctigenin as the active principle of *A. lappa*. In this article, we report the isolation of arctigenin as a natural product with potent inhibitory activity against PANC-1 tumor cell growth both *in vitro* and *in vivo*.

Materials and Methods

Materials. The dried seeds of *A. lappa* were commercially purchased from Tochimoto Tenkaido Co. (Osaka, Japan). The method of extraction and purification is described in detail in Results.

Cell lines and nutrient starvation. The human pancreatic cancer cell lines PANC-1, AsPC-1, PSN-1, BxPC-3, and the hepatoma cell line Alexander, obtained from the American Type Culture Collection, were maintained in DMEM (Nissui, Tokyo, Japan) supplemented with 10% FCS

Note: S. Awale and J. Lu contributed equally to this work.

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(Sigma, St. Louis, MO), 2% L-glutamine, 1% penicillin, and 1% streptomycin stock solutions; 20% FCS was used for the Capan-1 cell line. The medium was routinely changed every 3 days and the cells were passaged by trypsinization until they attained confluence. Nutrient starvation was achieved by culturing the cells in nutrient-deprived medium as previously described (5–7). Briefly, the composition of the nutrient-deprived medium was as follows: 265 mg/L $CaCl_2(2H_2O)$, 0.1 mg/L $Fe(NO_3)(9H_2O)$, 400 mg/L KCl, 200 mg/L $MgSO_4(7H_2O)$, 6,400 mg/L NaCl, 700 mg/L $NaHCO_3$, 125 mg/L NaH_2PO_4, 15 mg/L phenol red, 25 mmol/L HEPES buffer (pH 7.4), and MEM vitamin solution (Life Technologies, Inc., Rockville, MD); the final pH was adjusted to 7.4 with 10% $NaHCO_3$. For supplementation with glucose, D-glucose was added at a concentration of 1 mg/mL. For amino acid supplementation, stock solutions (200 mmol/L L-glutamine solution, MEM amino acids solution, and MEM nonessential amino acids solution; Life Technologies) were added at a concentration of 1%. The FCS (Sigma) used for medium supplementation was dialyzed thrice against large excesses of 0.9% NaCl before use.

Preferential cytotoxicity under nutrient-deprived conditions. The cytotoxicity assay was done with cell counting kit-8 from Dojindo Molecular Technologies, Inc., which is more sensitive than 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (8, 9). Briefly, PANC-1 cancer cells were seeded in 96-well plates (1×10^4 per well) and incubated in fresh DMEM medium at 37°C under a 5% CO_2/95% air for 24 hours. The cells were then washed with PBS and the medium was changed to either DMEM or nutrient-deprived medium followed by immediate addition of serial dilutions of the test samples. After 24-hour incubation, the cells were washed again with PBS, then 100 μL of DMEM medium with 10% WST-8 solution was added to the wells, and the plate was incubated for a further 2 hours. Then, the absorbance of the wells at 450 nm was measured. Because the absorbance is proportional to the number of viable cells in the medium, the viable cell number was determined using a previously prepared calibration curve (Dojindo Co., Kumamoto, Japan). The samples that were highly and selectively cytotoxic against the cancer cells in nutrient-deprived medium were subjected to further purification.

Western blot analysis. The proteins were separated by gel electrophoresis on a polyacrylamide gel containing 0.1% SDS and then transferred to nitrocellulose membranes. The membranes were blocked with PBS containing 5% (w/v) skim milk, washed with PBS containing 0.3% Tween 20 (Sigma), then incubated overnight at room temperature with Akt antibody and the phosphospecific (Ser^{473}) Akt antibody (New England Biolabs, Ipswich, MA) diluted with PBS. After washing, the membranes were incubated for 2 hours at room temperature with horseradish peroxidase–conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA) as the second antibody. The bands were detected with an enhanced chemiluminescence system (Amersham Biosciences UK Ltd., Buckinghamshire, United Kingdom).

Tumorigenesis in nude mice. Five-week-old female SPF/VAF BALB/cAn Ncrj-*nu/nu* mice were obtained from Charles River Japan (Yokohama, Japan), and 5×10^6 cancer cells in 0.3 mL DMEM were s.c. injected into the right side of the back of the animals. Two weeks later, 12 mice bearing tumors around 5 mm in diameter were randomly divided into a treatment group and a vehicle control group. Because arctigenin is poorly soluble in water, it was first dissolved in DMSO at 10 mg/mL and kept frozen until use. Just before administration, the stock solution of arctigenin was diluted in saline to a final concentration of 250 μg/mL (the final concentration of DMSO in saline is 2.5%). The mice were administered by i.p. injections of 0.2 mL of either arctigenin solution or vehicle on 6 days of the week until the end of the experiment. The tumor size and body weight were measured weekly and the tumor volume was calculated using the following formula:

$$\text{Tumor volume} = 4/3 \times 3.14 \times (L/2 \times W/2 \times W/2)$$

where L is the length of the tumor and W is its width.

Statistical analysis. All results were expressed as means ± SD. Statistical comparisons were conducted using Student's t test after ANOVA. The results were considered to be significant when $P < 0.05$.

Results

Purification and structure determination of the active principle extracted from the seeds of *A. lappa*. The dried seeds of *A. lappa* (10 kg) was chopped into small pieces and percolated overnight with CH_2Cl_2 (30 L × 3). The extracts were concentrated under reduced pressure to yield the CH_2Cl_2 extract (1.2 kg), which was chromatographed on silica gel (8 × 70 cm) eluted with hexane, and then with $CHCl_3$, to obtain the hexane-eluted fraction (900 g) and the $CHCl_3$-eluted fraction (800 g), respectively. The $CHCl_3$-eluted fraction was further subjected to silica gel chromatography (8 × 70 cm) using methanol-$CHCl_3$ (0-10%) to obtain three fractions (fraction 1, 275 g; fraction 2, 430 g; and fraction 3, 185 g). The preferential cytotoxic activity of each fraction against the PANC-1 cells was then evaluated as described in Materials and Methods, and fraction 2 (430 g) was found to be the most active with 100% preferential cytotoxicity under the nutrient-deprived condition at a concentration of 10 μg/mL. Fraction 2 was viscous oily and its TLC analysis indicated that it contained one major compound. To separate the major compound from the oily fraction, it was subjected to crystallization at room temperature for 2 days, which led the formation of colorless prism (60.0 g). The crystals showed $[\alpha]_D^{20}$ -28.4° (c = 0.077, $CHCl_3$). Its molecular formula was determined by HR-FABMS to be $C_{21}H_{24}O_6$ [m/z 373.4190 $(M + H)^+$]. Its IR spectrum indicated the presence of hydroxyl (3,550 cm^{-1}), lactone carbonyl (1,760 cm^{-1}), and phenyl (1,610 and 1,460 cm^{-1}) functional groups. Analysis of [^{1}H]nuclear magnetic resonance (NMR), [^{13}C]NMR, correlation spectroscopy, heteronuclear multiple-bond quantum coherence, heteronuclear multiple-bond correlation, and circular dichroism spectral data confirm its chemical structure to be arctigenin (Fig. 1).

Arctigenin showed preferential cytotoxicity against nutrient-deprived cells in a concentration- and time-dependent manner. As shown in Fig. 2*A*, PANC-1 is highly resistant to even extreme nutrient deprivation and can survive under this condition for >48 hours (5). In this study, this tolerance to nutrient deprivation was remarkably eliminated by arctigenin in a concentration-dependent as well as time-dependent manner. Cells exposed to arctigenin at a concentration of 0.01 μg/mL showed 100% cell death within 24 hours of starvation and this sensitivity to starvation was even more pronounced when arctigenin was added at a concentration of 1 μg/mL, at which 100% cell death was observed within 12 hours.

To investigate the precise mechanism of the cell death induced by arctigenin under nutrient-deprived conditions, we examined the mode of cell death using the Hoechst 33342/propidium iodide staining method, as described previously (6, 7). As shown in Fig. 2*B*, arctigenin at a concentration of 0.01 μg/mL induced nuclear condensation in the PANC-1 cells within 12 hours of incubation under nutrient-deprived conditions. Because propidium iodide staining of the nuclei in the absence of nuclear damage has been characterized as representing necrotic cell death, our results suggest that the nutrient-deprived PANC-1 cells exposed to arctigenin underwent necrotic cell death.

Arctigenin sensitizes the PANC-1 cells to death only under glucose-deprived conditions. We then examined the conditions under which arctigenin induced cell death in the PANC-1 cells by depriving the cell culture of glucose, amino acids, serum, or none of these. As shown in Fig. 3, arctigenin (0.01 μg/mL) caused cell death within 24 hours preferentially under the glucose-deprived condition, irrespective of the presence or absence of amino acids and/or serum.

Effect of arctigenin on the Akt pathway. In our previous work, kigamicin, pyrvinium pamoate, and troglitazone, which also

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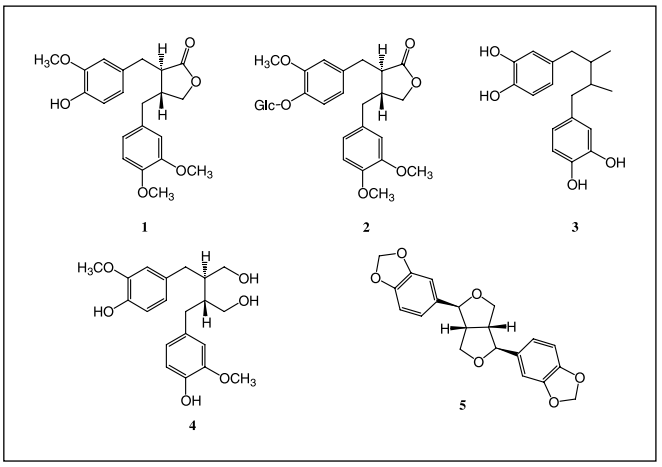

Figure 1. Structure of arctigenin (*1*), arctiin (*2*), nordihydroguaiaretic acid (*3*), secoisolariciresinol (*4*), and sesamin (*5*).

exhibited preferential cytotoxicity under the condition of glucose deprivation, were shown to inhibit Akt activation during glucose deprivation (5–7). Therefore, we examined whether arctigenin also influences Akt activation in the cells under nutrient-deprived conditions. As shown in Fig. 4, 0.1 μg/mL of arctigenin completely inhibited Akt phosphorylation stimulated by glucose deprivation. Furthermore, arctigenin was also found to suppress the activation of Akt stimulated by insulin and insulin-like growth factor-I (IGF-I) in a dose-dependent manner. These results clearly suggest that arctigenin inhibits the activation of Akt, which might be responsible for the cell death.

Effect of arctigenin on the survival of various cancer cell lines under nutrient-deprived conditions. To investigate whether the preferential cytotoxicity of arctigenin was limited to PANC-1 cells or was observed against various other cancer cells, we examined the effect of arctigenin on the survival of various cancer cell lines under nutrient-deprived conditions. As shown in Fig. 5, arctigenin diminished the tolerance of the cells to nutrient deprivation in all the tested cell lines, although to only a relatively small extent in KP-3 cells. Also, the AsPC-1 cell line was found to be the most sensitive, among all the cancer cell lines tested, to arctigenin under nutrient-deprived conditions. These results indicated that arctigenin was effective against many cancer cell lines.

Antitumor activity of arctigenin *in vivo*. To determine whether arctigenin also exerted antitumor activity *in vivo*, mice were inoculated with 5×10^6 PANC-1 cells s.c. on the back and then administered arctigenin or vehicle, as described in Materials and Methods. The body weight of the animals was monitored weekly (Fig. 6*A*) and no significant body weight loss was recognized in the treated group versus the vehicle control group at any time during the experimental period. The treatment was initiated from the 15th day by i.p. injection of the drug at the dose of 50 μg/mouse/d on 6 days of the week (or vehicle in the control group) until the 64th day. The tumor size was measured weekly. As is evident from the tumor growth curve shown in Fig. 6*B*, the tumor volume increased steadily in the control group, whereas the increase was significantly less prominent in the arctigenin-treated group. There was a significant difference in the tumor size between the arctigenin-treated group and the control group ($P < 0.05$), and the mean wet weight of the tumor was significantly higher in the control group than in the arctigenin-treated group (Fig. 6*C*). These data indicate that arctigenin also exerted antitumor activity *in vivo*. The mean tumor doubling time was 49 days in the arctigenin-treated group compared with 23 days in the control group. More importantly, no toxicity was observed in any of the animals at the dose used.

***In vitro* activity of arctigenin-related compounds.** To compare the antiausterity capability of arctigenin with that of other plant-derived lignans, we further tested the preferential cytotoxicity of arctiin, nordihydroguaiaretic acid, secoisolarirecinol, and sesamin. Interestingly, only arctigenin showed potent preferential cytotoxicity against PANC-1 cells under conditions of nutrient deprivation (Fig. 7), suggesting its unique antiausterity capability among the lignans. The glycoside arctiin is converted into arctigenin by the human intestinal flora (10, 11) after consumption, and thus the indirect role of the intestinal bacteria should also be considered in cancer chemoprevention activity of traditional medicines like *A. lappa*.

Discussion

Regulation of cell survival is crucial to the normal physiology of multicellular organisms and oxygen and nutrient supply to the cells and tissues is pivotal to the maintenance of their functions and integrity. However, the physiology of solid tumors differs from that of normal tissues in a number of important respects, the majority of which stem from the differences of the vasculature between

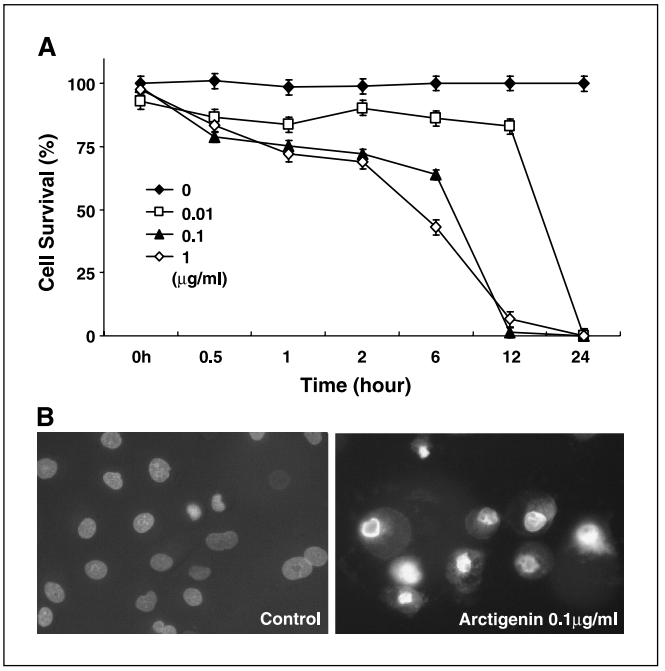

Figure 2. *A,* effect of arctigenin on cell survival in the PANC-1 cell line under nutrient-deprived conditions. Cells were seeded at a density of 1×10^4 per well in 96-well plates and incubated in fresh complete medium for 24 hours. The cells were then washed with PBS and the medium was changed to nutrient-deprived medium together containing graded concentrations of arctigenin. *Points,* mean from triplicate experiments. The cell number at the start of the starvation was considered to be 100%. The cell count was measured by the WST-8 cell counting kit method, as described in Materials and Methods. Data were analyzed with the Statview software (version 5.0 for Windows, SAS Institute, Inc., Cary, NC). *B,* arctigenin induced necrotic cell death under the nutrient-deprived condition. Panc-1 cells were cultured for 12 hours in nutrient-deprived medium, in the presence or absence of arctigenin (*Control*). After incubation, the cells were analyzed by the Hoechst 33342/propidium iodide staining method.

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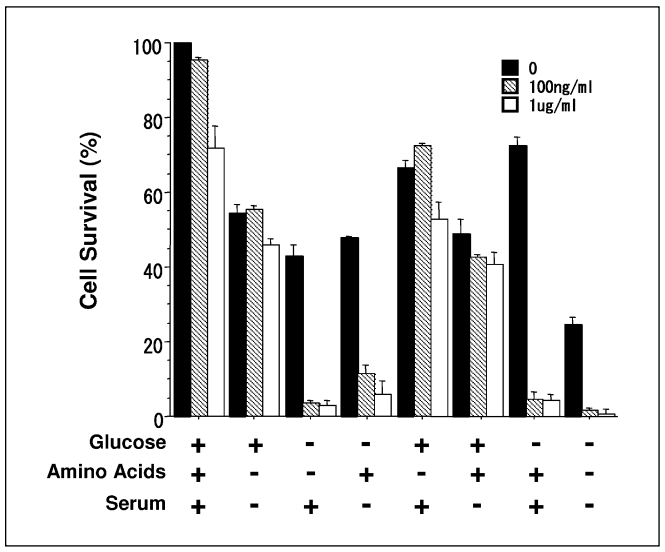

Figure 3. Nutrient deprivation and toxicity of arctigenin. Arctigenin (0.01 μg/mL) was added to the medium and the cell survival rate was examined 24 hours after the start of nutrient deprivation. Cell survival was examined by the WST-8 cell counting kit method. *Columns,* mean from triplicate experiments.

tumors and normal tissues. As compared with normal cells, tumor cells proliferate more rapidly and the oxygen as well as essential nutrition demand often exceeds the supply due to the unregulated growth of the cells caused by genetic and epigenetic alterations (12–14). Thus, tumor growth and progression largely depend on angiogenesis for functional blood supply, and antiangiogenic therapy was considered to be an attractive approach for cancer therapy. However, despite marked angiogenesis, large numbers of cells in a tumor are still in a state of oxygen and nutrient deprivation, partly because they proliferate more rapidly than normal endothelial cells and partly because the newly formed vascular networks are disorganized and, therefore, not optimally functional (12–14). Nevertheless, cancer cells have an inbuilt capability of surviving even under extreme conditions, such as that characterized by low nutrient and oxygen supply. This is particularly valid for highly aggressive tumors, such as pancreatic cancer, which are relatively hypovascular. Angiographic investigations have shown that most pancreatic cancers are hypovascular and that tumor vessels are noted only in a small number of cases (15–18). Ultrasonographic and computed tomographic studies have also shown that most pancreatic cancers receive poor blood supply despite considerable angiogenesis being noted histopathologically (19–21). The question, therefore, now, is how do pancreatic cancer cells survive and proliferate under conditions of severe hypoxia and nutrient deprivation? There must be some mechanism that facilitates the survival and proliferation of cancer cells under such extreme adverse environments.

In our earlier works, we hypothesized that tolerance to nutrient starvation might be a part of the biological response to insufficient blood supply and that targeting cancer cells under conditions of nutrition deprivation may be a novel approach in anticancer drug development. In this regard, we developed a novel screening methodology for anticancer agents based on this antiausterity strategy (6) and screened 500 medicinal plant extracts used in Japanese Kampo medicine. We found from this screening that a CH_2Cl_2-soluble extract of *A. lappa* exhibited preferential cytotoxicity against nutrient-deprived cancer cells at the concentration of 50 μg/mL. We thus carried out further bioassay-guided fractionation and isolation, which led to the isolation of arctigenin as the primary compound exhibiting preferential cytotoxicity against nutrient-deprived cells at a concentration of 0.01 μg/mL. Furthermore, the activity of arctigenin was found to be both concentration-dependent and time-dependent, and arctigenin was found to induce the necrosis of cancer cells under the nutrient-deprived condition in our study. We noticed that arctigenin was reported to induce apoptosis in colorectal adenoma and carcinoma cells (22). The disparity is possibly due to either the difference of the culture condition (the normal medium was used in that study whereas the nutrient-deprived medium was used in our study), or the difference of the concentration of arctigenin used (the concentration of arctigenin used in the previous study was hundreds of times higher than that used in our study). As nutrition deprivation seldom occurs in normal tissues, it may be considered that the antiausterity capability of arctigenin is specific to cancer cells. Interestingly, in our present study, arctigenin markedly inhibited the PANC-1 tumor formation in nude mice. Conventional screening strategies for agents exhibiting cytotoxicity use normal medium containing all the essential elements, such as glucose, amino acids, and serum, required for normal cell growth, and in this study, only natural product extracts having cytotoxicity were considered for further isolation, identification, and drug development. However, many compounds that exhibit cytotoxicity *in vitro* fail to show any significant cytotoxic activity *in vivo* in animal models. This is partly because the *in vitro* culture conditions of the cells fail to precisely mimic the actual *in vivo* environment of tumors. In this regard, nutrient-deprived conditions might closely resemble the natural austere environment of tumors. In the present study, whereas arctigenin did not exhibit any cytotoxicity against the PANC-1 cancer cell line under normal conditions (IC_{50} > 100 μg/mL), it exhibited preferential cytotoxicity under nutrient-deprived conditions; such anticancer activity *in vivo* indicates that our antiausterity strategy could be a feasible and novel approach for cancer therapy. Because the aim of anticancer treatment is to kill only the cancer cells, the therapeutic efficacy of anticancer agents under nutrient-deprived conditions may serve as a highly feasible approach for cancer prevention and treatment. It is noteworthy that the conventional chemotherapeutic drugs, such as 5-fluorouracil, Taxol, doxorubicin, cisplatin, and campothecin, show only weak

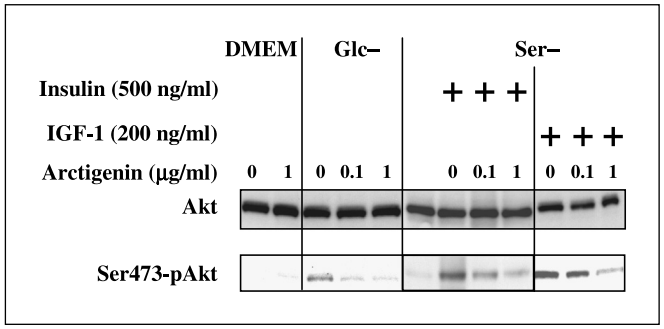

Figure 4. Effect of arctigenin on the activation of Akt as determined by Western blotting for the phosphorylated form (P-Akt Ser^{473}) in DMEM and under the glucose-deprived condition (*Glc–*) for 30 minutes. The effect of arctigenin on the activation of Akt stimulated by insulin and IGF-I was also investigated. The cells were seeded in a six-well plate for 24 hours in fresh normal medium, which was changed to serum-free (*Ser–*) medium followed by incubation overnight. Then, insulin and IGF-I were added at the concentrations indicated for 30 minutes; then all the cells were harvested, and the extracted protein was subjected to Western blot assay.

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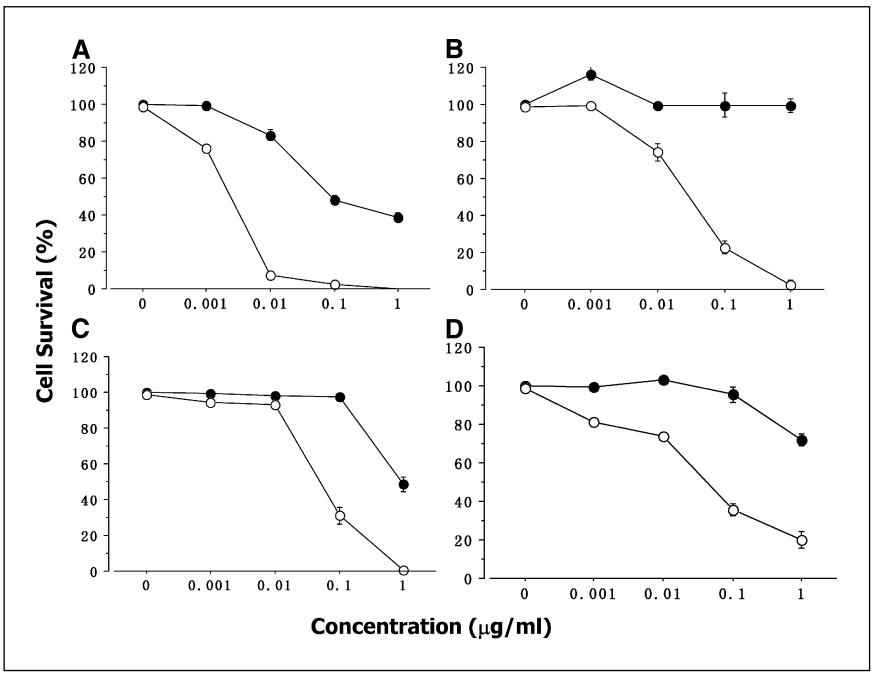

Figure 5. Effect of arctigenin on the survival of various cancer cell lines in normal DMEM (●) and nutrient-deprived medium, NDM (○). *A,* ASPC-1; *B,* BXPC-3; *C,* Alexander; *D,* PSN-1.

cytotoxicity against cancer cells cultured in nutrient-deprived media (6), which indicates that a different mechanism may underlie the antitumor activity of arctigenin against tumor cells growing in a low-nutrient, low-oxygen environment.

Arctigenin and arctiin have been reported to inhibit heterocyclic amine-induced colon, pancreatic, and hepatic carcinogenesis in rats, whereas 7,12-dimethylbenz(*a*)anthracene and 12-*O*-tetradecanoyl phorbol-13-acetate induced mouse skin carcinogenesis (23, 24). Arctigenin has also been reported to modulate immunologic responses (25) and to induce apoptosis in colon cancer cell lines (22). Therefore, the mechanism of antitumor activity of this agent may not be simple and the results of the present study suggest the involvement of the Akt pathway. Carcinogenesis is a complex process that includes both progressive acquisition of genetic

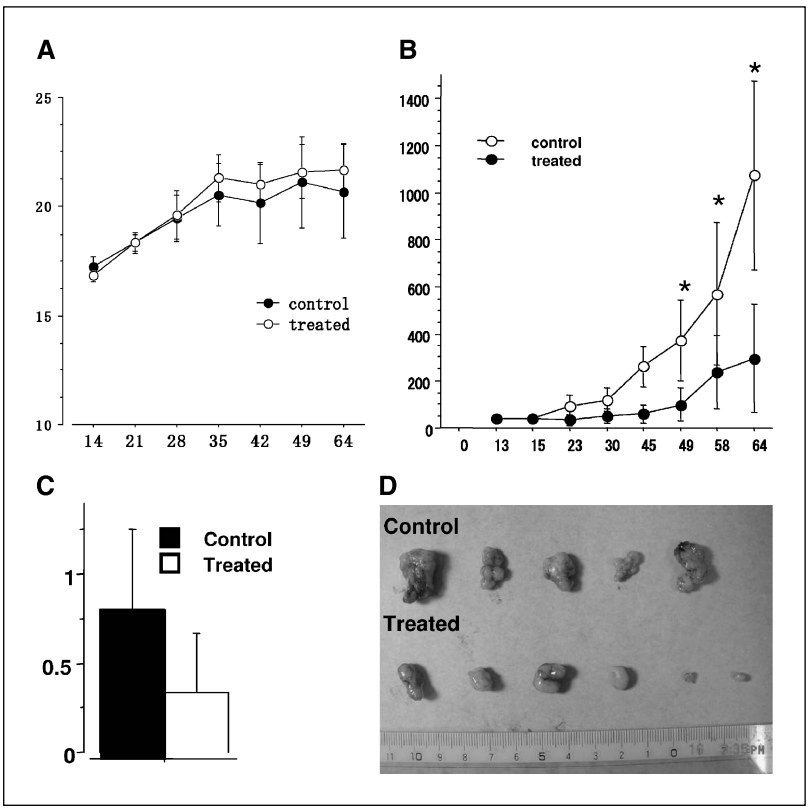

Figure 6. Effect of arctigenin on the growth of PANC-1 cells in nude mice. *A,* body weight of mice. ○, control group; ●, treated group. *Y axis,* body weight (g); *X axis,* time (day). *B,* the tumor volume in the mice. ○, control group; ●, treated group. *Y axis,* tumor volume (mm^3); *X axis,* time (day). *C,* wet weight of the tumor in the mice on the last day of the experiment. *Filled column,* control group; *clear column,* treated group. ($n = 6$). *Y axis,* wet weight of the tumor (g). *D,* photographs of the tumor after sacrifice on the last day.

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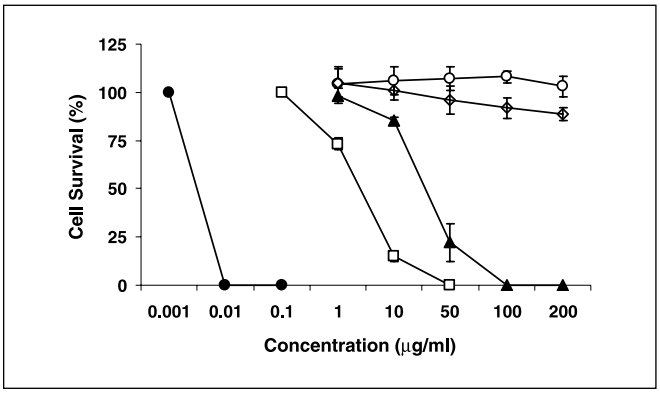

Figure 7. Effect of different lignans on the survival of PANC-1 cells in nutrient-deprived medium. ●, arctigenin; □, nordihydroguaiaretic acid; ▲, sesamin; ◇, secoisolarirecinol; ○, arctiin. Cells were seeded at a density of 1×10^4 per well in 96-well plates and incubated in completely fresh medium for 24 hours. The cells were then washed with PBS and the medium was changed to nutrient-deprived medium containing graded concentrations of arctigenin. The cell number at the start of the starvation was considered to be 100%.

alterations by cancer cells and reorganization of cancer tissues. During these complex processes, adaptation of the cancer cells to their tumor microenvironments is pivotal to tumor growth and progression. Therefore, it would be reasonable to consider agents that inhibit this adaptation of the cells to their microenvironment as chemopreventive agents. In this sense, inhibition of carcinogenesis by arctigenin might be related to the activity presently identified, namely, antiausterity.

Many lines of evidence show that Akt plays a critical role in the development, growth, and acquisition of therapeutic resistance of cancer cells. Inhibition of the Akt pathway might have therapeutic value in patients with cancer and this has formed the basis for widespread efforts to develop agents that inhibit Akt (26–28). Although the molecular and biochemical mechanism of austerity of cancer cells and the pharmacologic mechanisms of action of arctigenin are yet to be understood, inhibition of Akt phosphorylation by arctigenin under nutrient-deprived conditions found in this study might have relevance to its preferential cytotoxicity. Glucose deprivation occurs in many solid tumors as a consequence of the local decrease in blood supply and is an intrinsic variable of ischemia (12, 16, 19, 29). The severity of glucose deprivation may even be aggravated by a higher nutrient consumption in neoplastic tissues (30, 31). Under various stress conditions, including nutrient deprivation, PKB/Akt is involved in cell survival reactions. In our earlier study, we found that a high level of expression of PKB/Akt was associated with the tolerance of pancreatic cancer cells against nutrient deprivation (5). One mechanism by which Akt may promote cell survival is through the inhibition of a component of the cell death machinery. We have also found that PKB/Akt was strongly phosphorylated during glucose starvation, under both normoxic and hypoxic conditions. Therefore, inhibition of Akt phosphorylation by arctigenin may, at least in part, be responsible for the preferential cytotoxicity of arctigenin against nutrient-deprived cells. Actually, in our previous study, transfection of antisense RNA expression vector for Akt significantly diminished the tolerance of pancreatic cancer cell to nutrient deprivation, and LY294002, a representative phosphoinositide 3 kinase inhibitor, remarkably decreased cell survival of PANC-1 cell under nutrient-deprived condition (5). All evidences suggest that the inhibition of Akt activation by arctigenin have some relations with its preferential cytotoxicity to cancer cells, although the Akt pathway only might not be sufficient to explain the intricate mechanism for preferential cytotoxicity.

In conclusion, our findings provide a novel mechanistic insight into the actions of arctigenin in cancer therapy, based on antiausterity strategy, indicating again the feasibility of antiausterity strategy for cancer therapy.

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