Available online at www.sciencedirect.com

SCIENCE DIRECT

Gynecologic Oncology 99 (2005) 199 – 205

Gynecologic Oncology

www.elsevier.com/locate/ygyno

Potentiation of the radiation effect with genistein in cervical cancer cells

Catheryn M. Yashar [a], William J. Spanos [a], Douglas D. Taylor [a,b], Cicek Gercel-Taylor [a,b,*]

[a] Department of Radiation Oncology, University of Louisville School of Medicine, 511 South Floyd Street, Room 416, Louisville, KY 40202, USA
[b] Department of Obstetrics, Gynecology, and Women's Health, University of Louisville School of Medicine, 511 South Floyd Street, Room 416, Louisville, KY 40202, USA

Received 6 October 2004
Available online 3 August 2005

Abstract

Objective. Early stage cervical cancer is treated with surgery or radiation with equivalent results. Radiation is used for curative therapy of locally advanced disease and is combined with additional anti-tumor agents to improve control. We determined the potential role of genistein as a radiosensitizer for cervical cancer cells.

Methods. Human cervical cell lines (CaSki and ME180) were used. Sensitivity of cells to genistein, radiation and the combination was determined by colony assays. Western blotting was used to study the expression of cell-response-related gene products.

Results. Genistein results in the dose-dependent inhibition of all cell lines (2.5–40.0 μM). Effect of genistein on the radiosensitivity of the two cervical tumor cells was variable. Me180 cells were more sensitive at 20 and 40 μM of genistein. At 40 μM, less than 5% of Me180 cells survived the radiation (200–800 cGy). Potentiation of the radiation effect in CaSki cells was seen (500–800 cGy). The most significant enhancement of radiosensitivity was seen at 20 and 40 μM genistein at 500 and 800 cGy. G_2M arrest was demonstrated only in ME180 cells with genistein. There was significant inhibition of Mcl-1 by genistein that correlated with increase in radiosensitivity in Me180 cells. Activated pAKT (Thr 308) was inhibited with genistein and radiation in CaSki cells.

Conclusions. Genistein inhibits growth of cervical cancer cells. Genistein results in variable and significant enhancement of the radiation effect that may be partially mediated by G_2M arrest, Mcl-1 and activation of the AKT gene.

Keywords: Cervical cancer; Radiosensitivity; Genistein

Introduction

In the United States cervical cancer represents 2% of cancer deaths in women with approximately 15,800 diagnosed cases and 4800 deaths per year [1]. For early stage disease, cervical cancer can be treated with surgery or radiation with equivalent results. For locally advanced disease, however, radiation offers the only initial option for curative therapy. At the time of diagnosis approximately 45% are localized and an additional 34% have only regional spread. Both of these conditions are appropriately treated with radiation therapy [2]. To improve the local control and survival, radiation is best combined with additional anti-tumor agents. In 1998 five randomized phase III studies demonstrated an advantage in locally advanced cervical carcinoma when radiation was combined with chemotherapy [3–7]. Despite this, the progression-free survival is only 60–70% for combined stages, demonstrating room for additional improvement. All combined modality studies used cisplatin and several included 5-fluorouracil as well. Although the schedules and concentrations varied, doses used were those necessary for radiation sensitization but below that for full course therapy. More effective radiation with minimal toxicity to the host would be of value in the treatment of cervical cancer.

In this study, we tested whether genistein could augment the efficacy of radiation for the treatment of cervical cancer. Earlier studies have shown the effectiveness of genistein as a

* Corresponding author. Department of Obstetrics, Gynecology, and Women's Health, University of Louisville School of Medicine, 511 South Floyd Street, Room 416, Louisville, KY 40202, USA. Fax: +1 502 852 0881.
E-mail address: cgtayl01@gwise.louisville.edu (C. Gercel-Taylor).

0090-8258/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygyno.2005.07.002

radiosensitizer against prostate and esophageal tumor cells [8,9]. A recent report has demonstrated that genistein inhibits the growth of human cervical tumor cells in vitro [10]. Genistein also has been shown to inhibit growth of tumor cell lines from various malignancies including breast, endometrial, lung, melanoma, prostate, ovarian, head and neck squamous cell carcinoma, leukemia and lymphoma [10–16].

Genistein has a heterocyclic diphenolic structure and has been shown to inhibit tyrosine protein kinases, topoisomerase I and II, protein histidine kinase and 5α-reductase and induces G_2M cell cycle arrest in some cells that leads to cell growth inhibition [17,18]. There are two main classes of phytoestrogens, the isoflavones, found predominantly in soya beans, and lignans, which are found in a variety of foods including flaxseed, cereals, fruits and berries [19]. The major glycosides found in soya beans are daidzin, genistin and glycitin. These glucose-conjugated compounds are inactive estrogenically but upon consumption are hydrolyzed by mammalian enzymes and the gut microflora to form the active aglycone isoflavone compounds daidzein, genistein and glycetein. The coumestans constitute another class of phytoestrogens, of which coumestrol is the most studied. It is uncommon in the diet, found in clovers, soya bean and mung bean sprouts. Effect of genistein on disruption of the cell cycle may be the likely mechanism that results in the augmentation of cell killing induced by radiation. Genistein induces apoptosis in some, but not all, cells [20,21]. Mechanisms involved in genistein radiosensitization have not been elucidated; however, involvement of survival signals such as p42/p44 ERK and AKT/PKB has been proposed [9].

Since treatment of cervical cancer involves multi-modality treatments, new combined approaches need to be developed. Agents such as genistein have been extensively studied for their effect on tumor cells; thus, its potential use in the treatment of cervical cancer alone or in combination with other modalities may improve patient response with decreased toxicity. The question of whether genistein can be used therapeutically also includes studying the role of the other major isoflavone, daidzein, isolated from soy. These two components are widely consumed by patients and the normal population. We present the results of our study on the effect of genistein and daidzein on cervical cancer cell growth alone and in combination with radiation.

Materials and methods

Cell lines

CaSki and ME180 were obtained from American Type tissue Collection (ATCC, Gaithersburg, MD). Both cell lines are epithelial and grow as adherent cultures. CaSki cells produce the beta subunit of hCG and tumor-associated antigen (TAA). These cells contain integrated HPV-16 (600 copies/cell), as well as sequences related to HPV-18. ME-180 cells are p53, pRB positive and contain HPV DNA with greater homology to HPV-39 than HPV-18. CaSki cells express high affinity 56-kDa estrogen receptor. While binding of ^{3}H-estradiol is not demonstrated in ME-180 cells, immunoreactive estrogen receptor exists with Western blotting. Cell lines were cultured in RPMI medium supplemented with charcoal-treated 10% fetal bovine serum, sodium pyruvate, non-essential amino acids and antibiotic–antimycotic mixture (Sigma Chemical Co. St. Louis, MO) and incubated in a humidified CO_2 incubator at 37°C.

Cell treatments

Cells were treated with genistein or daidzein (stock solution 7.4 mM in DMSO and stored at −20°C) for 48 h continuously prior to radiation, and media were not changed during the procedure. For growth inhibition studies, cells were exposed to various concentrations continuously for the duration of the experiments.

Determination of cell survival

Radiation cell survival curves were generated using growth inhibition data obtained from clonogenic ability of single-plated cells (300/dish) in the presence of genistein, daidzein alone or with radiation. To determine clonogenic survival, increasing number of cells according to radiation dose (in triplicate) were cultured for 10–12 days, fixed with alcohol and stained with crystal violet. Colonies containing more than 50 cells were scored as survivors. Each experiment was performed in triplicate at least three times.

Radiosensitization was defined as when genistein increases the sensitivity of cells to radiation. Radiation ER (enhancement ratio) is defined as the ratio of surviving cells with radiation alone (200, 500 or 800 cGy) to the combination of radiation and genistein exposure-treated cells.

Radiation

Radiation doses were given as single fractions. For each radiation, dose-treated cultures and non-treated control cultures were irradiated simultaneously. Cells were grown and treated in 6-well plates and were irradiated with 4MV photons with a Phillips linear accelerator to 200, 500 or 800 cGy. The cells were irradiated with a vertical beam at 100 cm SSD with 1 cm of tissue-equivalent Lucite bolus with a field size of 39 × 39 cm and positioned at central axis with a machine dose rate of approximately 400 cGy min^{-1}.

Flow cytometry

Cells were treated with genistein for 48 h prior to staining with propidium iodide and fixing. Analysis was performed by a FACSCAN at the cell sorting facilities of the Brown Cancer Center at the University of Louisville.

Western Blot analysis

Cells were grown to confluency in 10-cm plates and harvested by trypsinization to isolate subcellular fractions for cytochrome *c* expression. Cells were collected by centrifugation at 600×*g* for 5 min at 4°C and resuspended in 1 ml of cytosol extraction buffer containing dithiothriol (DTT) and protease inhibitors and incubated on ice for 10 min. Cells were then homogenized by passing them through a 23-gauge needle five times to lyse plasma membranes. Nuclei and lysed membranes were removed by centrifugation at 700×*g* at 4°C for 10 min. The supernatant was transferred and centrifuged at 10,000×*g* for 30 min at 4°C. The supernatant following this spin was designated as the cytosolic fraction, and the pellet resuspended in mitochondrial extraction buffer (Apotech) was designated as the mitochondrial fraction. Total cell lysates were obtained from these cells lines by lysing cells in buffer containing SDS (20 mM Tris, pH 7.5, 100 mM NaCl, 50 mM KCl, 0.1 mM EDTA, 0.1 mM PMSF, 0.2% NP-40, 0.1% Triton X-100, 1% SDS, 10% glycerol). Protein determinations were performed on all samples by the Bio-Rad assay. Approximately 30 μg of the various samples was electrophoresed on 8–16% polyacrylamide gradient gels. The proteins were transferred to nitrocellulose, blocked with 5% non-fat dry milk and probed with antibodies to cytochrome *c*, Mcl-1, AKT and pAKT (ser 473 and Thr 308) (Santa Cruz Co. Santa Cruz, CA). Horseradish-peroxidase-labeled mouse antibody was used as the second antibody. Immunoreactive bands were visualized with ECL (Amersham, Boston, MA).

Statistical analysis

One-way ANOVA was used to compare the effects of different radiation and genistein doses.

Results

Effect of genistein and daidzein on cervical cancer cell growth

We demonstrate the inhibitory effect of genistein on two human cervical cancer cell lines (Fig. 1). Significant dose-dependent inhibition of growth was seen with genistein in both cell lines. All cells exhibited similar sensitivities at 40 μM with a 75% or more decrease in surviving colonies. At the higher concentration of 80 μm complete inhibition of growth was observed (data not shown). Me180 cells were more sensitive to the inhibitory effect of genistein with an LD_{50} of 11 μm, while CaSki cells had an LD_{50} of 24 μm. Since growth stimulatory effect of genistein (1 μM) was previously shown with MCF-7 breast cancer cells, we performed experiments at this concentration with cervical tumor cells. We did not observe any increased proliferation at this concentration, and no significant inhibition was observed (data not shown). While the lowest concentrations of 2.5 and 5.0 μM did not result in significant inhibition in CaSki cells, ME180 cells were inhibited at these levels of genistein. We also present data for the inhibition of cervical cancer cells with daidzein (Figs. 2a and 2b). Daidzein (40 and 80 μM) inhibited both CaSki and Me180 cells. Lower doses of daidzein did not result in significant growth inhibitory effect in either cell line. Thus, genistein, and to a lesser degree daidzein, inhibits growth of cervical cancer cells.

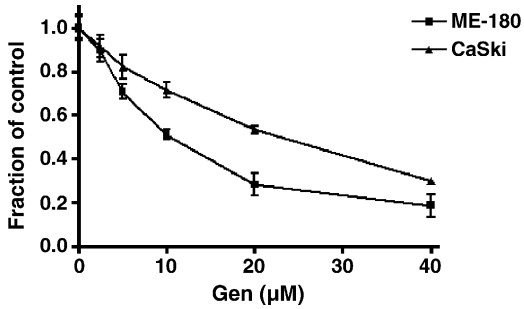

Fig. 1. Effect of genistein on the colony-forming ability of Me180 and CaSki cells. 300 cells were treated continuously with various doses for 12 days.

Effect of genistein and daidzein on the radiosensitivity of cervical cancer cells

The role of genistein and daidzein combined with radiation was investigated since improved radiation outcomes would be of tremendous value in the treatment of cervical cancer. Effect of genistein on the radiosensitivity of cervical tumor cells was variable (Figs. 3a and 3b). ME180 cells were more sensitive to the effects of genistein under the conditions of these experiments at 20 and 40 μM. At 40 μM, less than 5% of Me180 cells survived the radiation doses (200–800 cGy). On the other hand, potentiation of the radiation effect in CaSki cells with radiation is seen at 500 and 800 cGy doses with all genistein doses and appears to not be dose dependent. Radiation enhancement ratios are shown in Table 1. Enhancement of Me180 cells ranged from 1.6 to 91.1, with a substantial increase at 20 μM and above of genistein. In contrast, there was a slight but significant dose-dependent increase in enhancement ratios with CaSki cells demonstrating the radiosensitizing effect. Daidzein had no effect on radiosensitivity of CaSki and Me180 cells (data not shown). These experiments were performed by treating the tumor cells with genistein 48 h prior to radiation. After radiation the cells were cultured without genistein for the duration of the experiment before quantitation of colonies. Treatment period with genistein is significant in that other investigators have shown that maximum effect of genistein radiosensitization is seen with continuous exposure after radiation [22]. This may also be true in our case; however, significant differences are seen with exposure to genistein prior to radiation. We demonstrate that genistein acts as a radiosensitizer in vitro for cervical tumor cells.

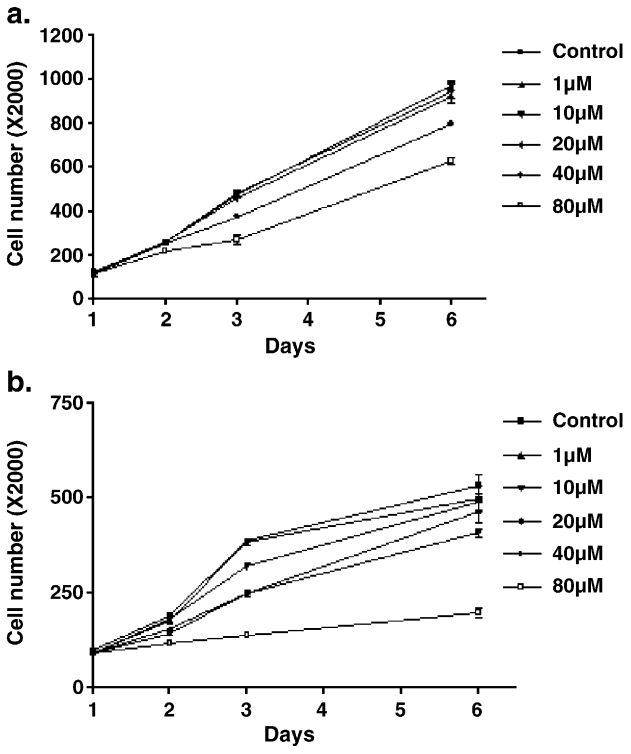

Fig. 2. (a and b) Effect of daidzein on cell growth of CaSki (a) and Me180 (b) cells. Cell counts were obtained daily for 6 days under constant exposure to varying concentrations of daidzein.

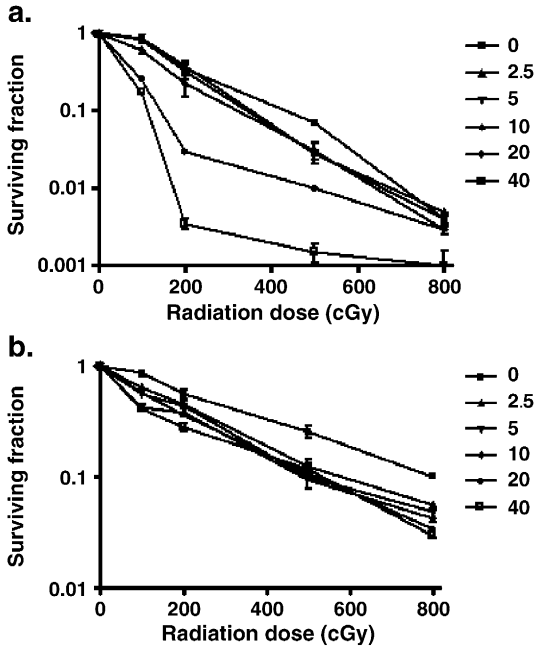

Fig. 3. (a and b) Effect of genistein on the radiosensitivity of cervical cancer cells. Colony formation was used to determine the effect of 48 pretreatment with various doses of genistein on radiosensitivity in Me180 (a) and CaSki (cells).

Table 1
Radiation enhancement ratios defined as survival with radiation and genistein compared to radiation for Me180 and CaSki cells

Genistein (μM)					
Cells	2.5	5.0	10.0	20.0	40.0
Me180	1.6	1.9	3.2	16.6	91.1
	(1.1–2.7)	(1.3–3.3)	(2.0–4.4)	(3.5–41.2)	(4.0–350.0)
CaSki	1.7	2.1	2.4	2.9	3.9
	(1.1–2.3)	(1.2–3.1)	(1.2–3.7)	(1.9–4.5)	(3.0–5.8)

Results are shown as average and range at 0–800 cGy.

Effect of genistein on the cell cycle

In order to understand the mechanisms involved in the increased radiation sensitivity seen with genistein, the effect of pretreatment with genistein on the cell cycle was studied. Our data demonstrate that the in vitro effect of genistein on cervical tumor cells includes G_2M arrest, specifically with Me180 cells (Table 2). As G_2M is the most sensitive phase of the cell cycle, it is reasonable to propose that this effect of genistein is at least partially involved in the radiosensitization effect. A similar effect of genistein is not seen in CaSki cells. These experiments were performed with 48-h genistein pretreatment. When CaSki cells were incubated with genistein for 3 days prior to radiation with genistein, their sensitivity to radiation was increased; however, cell cycle changes resulting in G_2M arrest were not seen.

Effect of genistein on the expression of cytochrome c, Mcl-1 and AKT

Additional experiments were directed at the study of cell-death-related gene products with genistein in cervical cancer cell lines. In both cell lines a dose-dependent induction of cytochrome *c* suggests the involvement of the apoptotic pathway (Fig. 4). Levels of induction are not significantly different between cell lines when the fold increase in cytochrome *c* is calculated based on the background levels. Subsequently, we studied the effect of genistein on the levels of Mcl-1 and AKT. Study of Mcl-1 expression correlated well with the increased radiosensitivity of cervical cancer cells (Fig. 5). The most sensitive, Me180 cells, had a significant decrease of Mcl-1 with genistein in a dose-dependent manner. Addition of radiation resulted in a further decrease of Mcl-1 in Me180 cells. CaSki cells also

Table 2
Cell cycle analysis of genistein-treated cells

	CaSki (control/48 h genistein treatment)	Me180 (control/48 h genistein treatment)
G_1 (%)	78.28/76.70	76.04/32.64
S (%)	5.48/13.60	6.80/9.14
G_2M (%)	16.24/9.70	17.16/58.22

CaSki and Me180 cells were treated for 48 h prior to propidium iodide staining and FACS analysis.

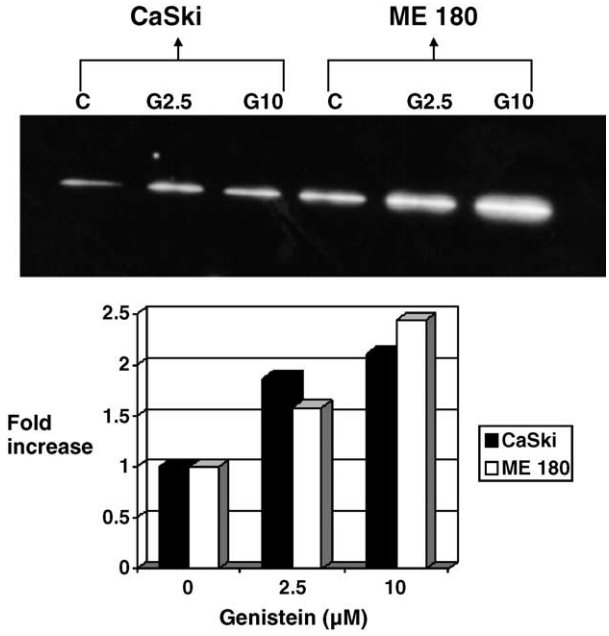

Fig. 4. Cytochrome *c* induction in CaSki and Me180 cells following 48-h treatment with 2.5 and 10 μM of genistein. Fold increase was calculated from scanning density of treated samples compared to controls.

demonstrated a dose-dependent decrease of Mcl-1 expression with genistein and radiation.

Total AKT levels were decreased in Me180 cells after an initial increase with 10 μM genistein treatment and 3 h after radiation treatment only (Fig. 6). Activated AKT levels were similar in that genistein and radiation resulted in decreased levels that may contribute to the sensitivity of Me180 cells. Similarly, total AKT levels in CaSki cells were decreased in the presence of genistein. However, there was an increase in AKT levels with radiation treatment in the presence of genistein. Activated AKT (Ser 473) was decreased in the presence of 40 μM genistein and no other significant decrease was seen with genistein and/or radiation in CaSki cells. pAKT (Thr 308) levels were significantly decreased with genistein (40 μM) and radiation.

Discussion

Our data demonstrate that genistein results in dose-dependent inhibition of the cervical cell lines (2.5–40 μM). In addition, genistein has radiosensitizing effect on the CaSki and Me180 cells. Daidzein had no effect on radiosensitivity of CaSki and Me180 cells. CaSki and Me180 cells were utilized in this study because they represent two spectra of HPV infection. CaSki cells contain high risk HPV and Me180 has low risk HPV39. Also, p53 status of these cells is different such that Me180 cells are p53 positive and CaSki cells have p53 inactivated by HPVE6. Our initial experiments were performed with ovarian tumor cell lines [22]. We utilized five ovarian cancer cell lines and demonstrated various levels of inhibition with genistein and daidzein, genistein being the more effective of the two. Similar to previously published data, we demonstrated that daidzein results in the apoptosis of ovarian cancer cells, but genistein exerts a cytostatic/cytotoxic effect depending on the concentration and exposure time [23]. We also demonstrated that genistein, when combined with chemotherapeutic drugs used to treat ovarian cancer, results in an additive effect with cisplatin, paclitaxel and topotecan.

Previous studies have shown that the effects of genistein are dose and time dependent. Genistein at 15 μM caused a significant inhibition in DNA synthesis, cell growth and colony formation in the range of 40–60% and potentiated the effect of 200–300 cGy photon or 100–150 cGy neutron radiation in prostate cancer cells [8]. Cell growth inhibition induced by genistein alone or combined with radiation is dependent on the presence of genistein during the 10-day incubation of the colony assay [8]. Much milder effect was seen if genistein was not present following radiation. This suggests that continuous exposure is needed for maximal effect. Doses of photon (200–300 cGy) or neutron (100–150 cGy) radiation, showing optimal augmentation of cell killing when combined with 15 μM genistein, are the normally used fractionated doses of therapeutic radiation. It should be noted that concentrations of genistein in the range of 5–15 μM are considered mild doses and have shown no toxic effect on normal breast epithelial cells in vitro [24]. In our studies, Me180 cells were more sensitive at 20 and 40 μM of genistein. At 40 μM, less than 5% of Me180 cells survived the radiation (200–800 cGy). In addition, potentiation of the radiation effect in CaSki cells was seen (500–800 cGy). Genistein treatment resulted in G_2M arrest only with ME180 cells.

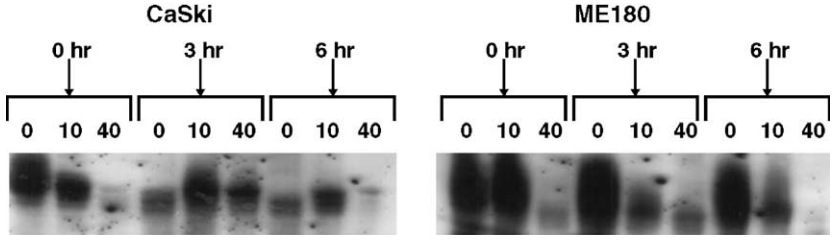

Fig. 5. Western blot analysis of the effect of genistein (10 and 40 μM) and radiation (500 cGy) of CaSki and Me180 cells on Mcl-1 expression. Radiation effect was studied at time = 0, 3 and 6 h after treatment.

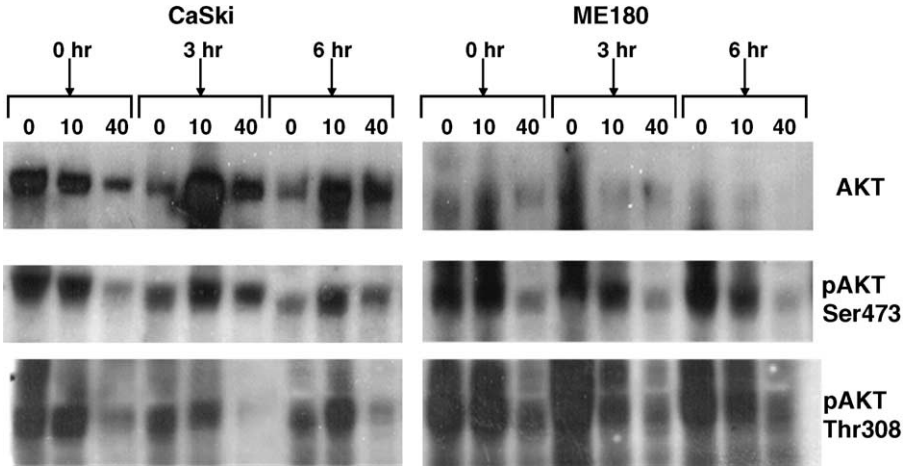

Fig. 6. Western blot analysis of the effect of genistein (10 and 40 μM) combined with radiation on AKT and pAKT (ser 473 and Thr 308) in CaSki and Me180 cells. Radiation dose used was 500 cGy, and cells were harvested at 0, 3 and 6 h after treatment.

We studied constitutive levels of AKT and the phosphorylated form as a mechanism of radiosensitization. P13K/AKT pathway regulates fundamental cellular functions such as cell growth, survival and movement. Full activation of AKT requires phosphorylation at two sites, one within the activation loop (T308 for AKT1) and one within the C-terminus (S473 for AKT1). Activation of AKT in response to cellular stress may be a generalized protective mechanism activated by the cell to escape death [25]. Once active, AKT controls cellular functions through phosphorylation of downstream genes responsible for cellular response. The aggregate results from recent studies indicate that the ability of chemotherapy to inhibit AKT activity correlates well with the cytotoxic effects of these agents. Although most reports have shown that chemotherapy decreases AKT activity, there is also evidence that chemotherapeutic agents can increase AKT activity [26,27]. In most cases, increases in AKT activity in response to chemotherapy are transient and are followed by subsequent decreases in activity [26,27]. Point of when AKT activity was assayed may determine the expression patterns. With the cervical cells studied here, constant decrease in AKT was associated with the most sensitive Me180 cells. CaSki cells that were more radioresistant than Me180 cells even in the presence of genistein only had significant reduction of pAKT (Thr 308) levels. This is in contrast to Me180 cells that had decreased levels of both activated forms of AKT.

Immunohistochemical studies demonstrated that Mcl-1, but not Bcl-2 or Bcl-x_L, has higher levels in human cervical cancer tissue than in normal cervix tissue [28]. It appears that Mcl-1 expression is more widespread in cervical cancer than Bcl-2 since expression is mutually exclusive. pI3-k/AKT-dependent pathway results mainly from IL-6-induced Mcl-1 up-regulation in human cervical cancer cells. Signaling pathways leading to the Mcl-1 induction may vary according to the type of cell models or stimuli employed. The PI3-k/AKT signaling pathway has recently attracted extensive attention because it may affect multiple cellular processes including increasing cellular proliferation, inhibiting apoptosis, upregulation of Mcl-1 and facilitating tumorigenesis.

There is no epidemiologic data to suggest that patients in populations who ingest soy products routinely have unfavorable outcomes with radiation treatment. Concentrations of genistein to achieve most of the in vitro effects are higher than the 3–4 μM reported to be achievable in the plasma of individuals who consume large amounts of soy products. Data supporting the risk lowering potential of soy protein containing isoflavones are from Asian populations who consume as much as 80 mg of total isoflavones per day with additional studies reporting 39.4 mg/day and 47.4 mg/day. Regular dietary intake results in the nanomolar quantities of genistein. Studies have reported measurements of physiologic concentrations of genistein in the range of 276 nM to 6 μM, depending on the study subjects and the isoflavone source. A recent report has shown that up to 27.5 μM of genistein in human plasma can be achieved after receiving genistein supplement at a dose of 16.0 mg/kg [29,30]. Thus, concentrations used in our experiments can be obtained by oral administration of genistein with no toxicity. While we do not know if normal concentrations of genistein are adequate therapeutically, our current findings suggest that in vivo studies can be used to answer these types of questions. In addition, differences in the cellular responses to the effects of radiation combined with genistein are of interest and worthy of further investigation.

Acknowledgment

We thank Bo Mason for technical assistance.

References

[1] Wingo PA, Tong T, Bolden S. Cancer statistics. CA Cancer J Clin 1995;45:8.

[2] Leibel SA, Phillips TL. Oncology: carcinoma of the uterine cervix. Textbook of Radiation. WB Saunders Company. p. 799.

[3] Whitney CS, Sause W, Bundy BN, et al. Randomized comparison of fluorouracil plus cisplatin versus hydroxyurea as an adjunct to radiation therapy in stages IIB-IVA carcinoma of the cervix with negative para-aortic lymph nodes: a gynecologic oncology group and southwest oncology group study. J Clin Oncol 1999;16:1339–48.

[4] Morris M, Eifel PJ, Lu J, et al. Pelvic radiation with concurrent chemotherapy versus pelvic and paraaortic radiation for high-risk cervical cancer: a randomized therapy oncology group clinical trial. N Engl J Med 1999;340:1137–43.

[5] Rose PG, Bundy BN, Stehman FB, et al. Concurrent cisplatin-based chemoradiation improves progression-free and overall survival in advanced cervical cancer: results of a randomized gynecologic oncology group study. N Engl J Med 1999;340:1144–53.

[6] Keys HM, Bundy BN, Stehman FB, et al. A comparison of weekly cisplatin during radiation therapy versus irradiation alone, each followed by adjuvant hysterectomy in bulky stage 1B cervical carcinoma: a randomized trial of the gynecologic oncology group. N Engl J Med 1999;340:1154–61.

[7] Peters WA III, Liu PY, Barrett RJ, et al. Cisplatin and 5-fluorouracil plus radiation therapy are superior to radiation therapy as adjunctive in high-risk early-stage carcinoma of the cervix after radical hysterectomy and pelvic lymphadenectomy: report of a phase III intergroup study. Soc Gynecol Oncol 1999;72:443 [Abstract].

[8] Hillman GG, Forman JD, Kucuk O, et al. Genistein potentiates the radiation effect on prostate carcinoma cells. Clin Cancer Res 2001; 7:382–90.

[9] Akimoto T, Nonaka T, Ishikawa H, et al. Genistein, a tyrosine kinase inhibitor, enhanced radiosensitivity in human esophageal cancer cell lines in vitro: possible involvement of inhibition of survival signal transduction pathways. Int J Radiat Oncol Biol Phys 2001;50(1): 195–201.

[10] Wang SY, Yang KW, Hsu YT, Chang CL, Yang YC. The differential inhibitory effects of genistein on the growth of cervical cancer cells in vitro. Neoplasma 2001;48:227–33.

[11] Hawryleciwz EJ, Huang HH, Blair WH. Dietary soybean isolate and methionine supplementation affect mammary tumor progression in rats. J Nutr 1991;121:1693–8.

[12] Masai M, Abe K, Akimoto S, Yatani R, Shimazaki J. Argyrophilic nucleolar organizer regions in benign hyperplastic and cancerous human prostates. Prostate 1992;20:1–13.

[13] Chiusa L, Galliano D, Formiconi A, Di-Primio O, Pich A. High and low risk prostate carcinoma determined by histologic grade and proliferative activity. Cancer 1997;79:1956–63.

[14] Shirai T, Imaida K, Iwasaki S, Mori T, Tada M, Ito N. Sequential observation of rat prostate lesion development induced by 3,2′-dimethyl-4-aminobiphenyl and testosterone. Jpn J Cancer Res 1993; 84:20–5.

[15] Tanaka T, Takeuchi T, Nishikawa A, Takami T, Mori H. Nucleolar organizer regions in hepatocarcinogenesis induced by *N*-2-fluorenylacetamide in rats: comparison with bromodeoxyuridine immunohistochemistry. Jpn J Cancer Res 1989;80:1047–51.

[16] Lamartiniere CA, Moore JB, Brown NM, Thompson R, Hardin MJ, Barnes S. Genistein suppresses mammary cancer in rats. Carcinogenesis 1995;16:2833–40.

[17] Markovits J, Linassier C, Fosse P, et al. Inhibitory effects of the tyrosine kinase inhibitor genistein on mammalian DNA topoisomerase II. Cancer Research 1989;49:5111–7.

[18] Fotsis T, Pepper M, Adlercreutz H, Hase T, Montesano R, Schweiggerer L. Genistein, a dietary ingested isoflavonoid, inhibits cell proliferation and in vitro angiogenesis. J Nutr 1995;125:790s–7s.

[19] Setchell KDR. Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. Am J Clin Nutr 1998;68(6 Suppl.):1333S–46S.

[20] Upadhyay S, Madhavi N, Chinni SR, Alhasan S, Miller F, Sarkar FH. Differential sensitivity of normal and malignant breast epithelial cells to genistein is partly mediated by $p21^{WAF1}$. Clin Cancer Res 2001;7: 1782–9.

[21] Dampier K, Hudson LM, Manson MM, Walker RA, Gescher A. Differences between human breast cell lines in susceptibility towards growth inhibition by genistein. Br J Cancer 2001;85:618–24.

[22] Gercel-Taylor C, Feitelson AK, Taylor DD. Inhibitory effect of genistein and daidzein on ovarian cancer cell growth. Anticancer Research 2004;24:795–800.

[23] Shao Z-M, Alpaugh ML, Fontana JA, Barsky SH. Genistein inhibits proliferation similarly in estrogen receptor-positive and negative human breast carcinoma cell lines characterized by $p21^{WAF1/CIP1}$ induction, G_2-M arrest and apoptosis. J Cell Biochem 1998;69:44–54.

[24] Li Y, Upadhyay S, Bhuiyan M, Sarkar FH. Induction of apoptosis in breast cancer cells MDA-MB-231 by genistein. Oncogene 1999;18: 3166–72.

[25] West KA, Castillo S, Dennis PA. Activation of the P13k/Akt pathway and chemotherapeutic resistance. Drug Resist Updates 2002;5:234–48.

[26] Clark AE, West K, Streicher S, Dennis PA. Constitutive and inducible Akt activity promotes resistance to chemotherapy, trastuzumab, and tamoxifen in breast cancer cells. Mol Cancer Ther 2002; 1:707–17.

[27] Yakes FM, Chinratanalab W, Ritter CA, King W, Seelig S, Arteaga CL. Herceptin-induced inhibition of phosphytidylinositol-3 kinase and Akt is required for antibody-mediated effects on p27, cyclin D1, and anti-tumor action. Cancer Res 2002;62:4132–41.

[28] Wei LH, Kuo ML, Chen CA, et al. The anti-apoptotic role of interleukin-6 in human cervical cancer is mediated by up-regulation of Mcl-1 through a PI3-K/Akt pathway. Oncogene 2001;20:5799–809.

[29] Sarkar FH, Li W. The role of isoflavones in cancer chemoprevention. Front Biosci 2004;9:2714–27.

[30] Busby MG, Jeffcoat AR, Bloedon MA, et al. Clinical characteristics and pharmacokinetics of purified soy isoflavones: single-dose administration to healthy men. Am J Clin Nutr 2002;75:126–36.