Original Research

Genistein downregulates *de novo* lipid synthesis and impairs cell proliferation in human lung cancer cells

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Abstract

Cancer cells require high levels of lipid synthesis to produce structural, signaling and energetic lipids to support continuous replication. We and others have reported that constitutively increased lipogenesis, mainly by the tandem activation of acetyl-CoA carboxylase, fatty acid synthase and stearoyl-CoA desaturase-1 (SCD1), is critical to sustain the biological features of cancer cells, making this metabolic pathway a potential anticancer target for nutritional and pharmacological interventions. Isoflavones are biologically potent botanical compounds that possess clear antilipogenic and anticancer properties; however, the regulatory effects of these nutraceutical agents on lipid biosynthesis in cancer cells are still not well understood. Here we show that genistein, an isoflavone abundant in soybeans, decreased the levels of SCD1 protein in H460 human lung adenocarcinoma cells, consequently reducing the rate of biosynthesis of oleic acid as well as its presence in cancer cell lipids. Moreover, genistein promoted a marked reduction in *de novo* synthesis of major phospholipids, triacylglycerol and cholesterolesters. Finally, cancer cells treated with genistein displayed a dramatic reduction in cell proliferation as a result of a blockade in cell cycle progression through G_2/M phases. As a whole, our data suggest that, by globally downregulating lipid biosynthesis, genistein suppresses cancer cell growth, emphasizing the relevance of this botanical compound as a potential therapeutic agent against lung cancer, a disease for which therapeutic choices remain limited.

Keywords: lipid synthesis, cancer cells, cell proliferation, soy isoflavones, lung cancer

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Introduction

A recent study indicates that lung cancer is the main cause of death by cancer in the USA, with a ~16% chance of survival for new cases.[1] The lack of successful therapies to treat lung cancer and other highly lethal malignancies calls for the identification of new potential therapeutic targets. A notable feature of cancer cells is the uncontrolled rate of cellular proliferation. Due to unremitting mitogenesis, cancer cells require high levels of metabolic fuels and structural biomolecules for the rapid synthesis of new membranes and organelles for daughter cells.[2,3] Active lipid metabolism is a key to sustain these requirements, particularly the pathways of lipid biosynthesis, making these metabolic routes potential anticancer targets. Stearoyl-CoA desaturase 1 (SCD1), a key lipogenic that catalyzes the conversion of saturated fatty acids (SFAs) into monounsaturated fatty acids (MUFAs), has been found upregulated in several cancer cells and experimental tumors.[4] Importantly, it has been shown in cancer cells that, by controlling the rate of MUFA biosynthesis, SCD1 modulates the fatty acid profile of major lipids and determines the overall rate of lipogenesis.[5–7] We have also demonstrated that the inhibition of SCD1 causes a marked decrease in the rate of cellular proliferation in cancer cells by inducing cell cycle arrest in the G_0/G_1 phase, as well as by inducing SFA-mediated lipoapoptosis.[6–9] Remarkably, loss of SCD1 activity delays tumor formation and prevents further tumor growth.[7] SCD1 appears to control the rate of mitogenesis and tumorigenesis by simultaneously modulating the provision of MUFA for acylation reactions and by regulating the activity of signaling pathways, such as Akt and AMP-activated kinase.[10] Altogether, these findings suggest that inhibition of SCD1 may prove useful as a treatment for cancer.

For centuries, plant-derived compounds have been used to treat and prevent a variety of diseases.[11] Among these botanical compounds, isoflavones have captured the attention of several areas of pathology and therapeutics since these molecules exert a variety of beneficial effects in the organism, particularly in the prevention and treatment of

several metabolic diseases such as cardiovascular diseases, osteoporosis and cancer.[12] The most significant sources of isoflavones are soybean and its derived products. The isoflavone family comprises a group of several compounds including genistein, daidzein, genistin, formononentin and biochanin A.[13] Genistein is considered the most biologically active and best studied isoflavone. This compound has been reported as an antagonist of estrogen receptors, and shown to abolish the activity of growth factor-related tyrosine kinases such as epidermal growth factor (EGF) receptors.[12,14] Recent findings on the regulatory role of genistein in glucose and lipid metabolism have dramatically increased the interest in elucidating the metabolic effects of this compound. Interestingly, isoflavones, particularly genistein, have been reported to modulate lipid synthesis animal tissues and cultured cells. Added to isolated rat adipose cells, genistein depresses lipid synthesis.[15] In hepatoma cells, genistein downregulates the expression of SREBP-1, a master regulator of lipogenesis, and consequently the expression of fatty acid synthase and SCD1.[16] The effect of isoflavones on the rate of lipid synthesis and potential modifications of fatty acid distribution in cancer cells have not been fully described.

In the present study, we show that genistein suppresses *de novo* lipid biosynthesis in H460 human lung adenocarcinoma cells. As part of its antilipogenic effect, genistein decreases SCD1 expression and activity, therefore promoting marked changes in the MUFA/SFA ratio and in the segregation of exogenous fatty acids into cell lipid pools. Furthermore, genistein reduced the proliferation of cancer cells by blocking the progression of cell cycle in the G_2/M phase. Altogether, our findings suggest that genistein, by downregulating the highly active lipogenic pathway in cancer cells, may display its anticancer effects. Our data add more evidence in support of a potential use of genistein and similar antilipogenic nutraceuticals as therapeutic alternatives for treating lung cancer and other highly lethal malignancies.

Materials and methods

Materials

AG01518 normal human skin fibroblasts were obtained from Coriell (Camden, NJ, USA). H460 human lung adenocarcinoma cells were from ATCC (Manassas, VA, USA). Cell culture media and other culture reagents were from Invitrogen Life Technologies (Carlsbad, CA, USA). [1-^{14}C]stearic acid and [1-^{14}C]sodium acetate were from American Radiolabeled Chemicals, Inc (St Louis, MO, USA). Genistein, daidzein, ultrafiltered fetal bovine serum (FBS), fatty acid-free bovine serum albumin (BSA), mouse anti-β-actin monoclonal antibody, anti-mouse IgG peroxidase conjugate, phosphatase and protease inhibitor cocktail were purchased from Sigma (St Louis, MO, USA). Cell culture supplies, silica gel 60 chromatography plates and analytical-grade solvents were from Fisher Scientific (Morris Plains, NJ, USA). Polyclonal anti-SCD1 antibody was a generous gift from Dr Jean-Baptiste Demoulin (Université Catholique de Louvain, Belgium).

Cell culture

Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, penicillin (100 U/mL), streptomycin (10 μg/mL), 1% non-essential amino acids and 1% minimum essential medium vitamin solution (growing medium), at 37°C, 5% CO_2 and 100% humidity.

Cell proliferation assay

Cell proliferation rate was determined by crystal violet assay.[10] Cells were incubated with 100 μmol/L genistein, 100 μmol/L daidzein or dimethyl sulfoxide (DMSO) vehicle for 48 h. Cells were incubated with the isoflavones for 48 h in order to allow for at least one population doubling.

Determination of cell cycle distribution

In order to determine the distribution of cell populations in different phases of cell cycle, H460 cells were treated with either 100 μmol/L genistein or DMSO vehicle for 48 h. At the end of incubations, cells were collected and treated with 50 μL RNaseI (1 mg/mL) and stained with 5 μL propidium iodide (1 mg/mL). The percentage of cells in cell cycle phases was analyzed by fluorocytometry.

SCD activity and *de novo* lipid synthesis

Δ9-fatty acyl desaturase activity in whole H460 cells was determined as previously described.[10] Briefly, subconfluent cell monolayers were incubated with the specified concentration of genistein or DMSO vehicle in 10% FBS DMEM for 48 h. Six hours prior harvesting, cells were pulsed with [^{14}C]stearic acid (0.25 μCi/60 mm Petri dish) in culture medium containing 0.5% BSA. Total cellular lipids were extracted according to Bligh and Dyer[17] and transesterified with BF_3 in methanol for three hours at 64°C under nitrogen atmosphere. The methyl esters were separated on silver nitrate-thin-layer chromatography (TLC) following the procedure described by Wilson and Sargent.[18] The radiolabeled stearic and oleic acids were detected with a Storm scanner (Molecular Dynamics, GE Healthcare BioSciences, Pittsburgh, PA, USA) and its optical density quantified with Imagequant software. For *de novo* lipid synthesis, cells were treated with genistein or DMSO for 48 h and incubated for the last 16 h with [^{14}C]sodium acetate. Cellular lipids were extracted as described above and individual phospholipids and neutral lipids were separated by a one-dimensional, two solvent system TLC, as described in Scaglia and Igal.[6] Radiolabeled lipid spots on the TLC plate were detected and quantified as stated above. The amount of [^{14}C]tracer incorporated into lipids was normalized to cellular protein content of cells grown in parallel Petri dishes under similar experimental conditions.

Total cellular fatty acid composition

Total cellular lipids from H460 cancer cells treated with 100 μmol/L genistein or DMSO for 48 h were extracted as described above. After fatty acids from total lipids were

transesterified and methylated, the fatty acid methyl ester composition was determined by gas chromatography using a Varian 3800 GC (Varian Inc, Palo Alto, CA, USA), equipped with a DB-23 column (J&W Scientific Inc, Folsom, CA, USA) and flame ionization detector. Fatty acid methyl ester identification and response factors were determined using standard mixtures (NuChek Prep Inc, Elysian, MN, USA). Chromatographic peaks were identified by comparison of their retention times with those of pure fatty acid standards and percent distribution was calculated.

Determination of cellular protein

Total cellular protein content was measured by the Bradford method, using BSA as a standard.

Statistical analysis

Results from a representative experiment with at least three samples per experimental group are presented as means ± SD. Statistical significance of the data was determined by Student's *t*-test.

Results

Genistein impairs cell proliferation by blocking the progression of cell cycle

In agreement with previous reports indicating that isoflavones affect cancer cell growth,[19–21] we observed that genistein dramatically reduced the rate of proliferation of lung cancer cells (Figure 1a). Similar treatment produced no changes in the replication of normal human skin fibroblasts (data not shown), suggesting that genistein may differentially affect fast-proliferating cells. We also sought to determine whether the effects of genistein on cancer cell proliferation could be attributed to specific alterations in cell cycle progression. As shown in Figure 1b, incubation with genistein caused a significant arrest of cell cycle, with a reduction of cell populations in the G_0/G_1 phase, a drastic decrease in the S phase and an accumulation of cells in the G_2/M phase.

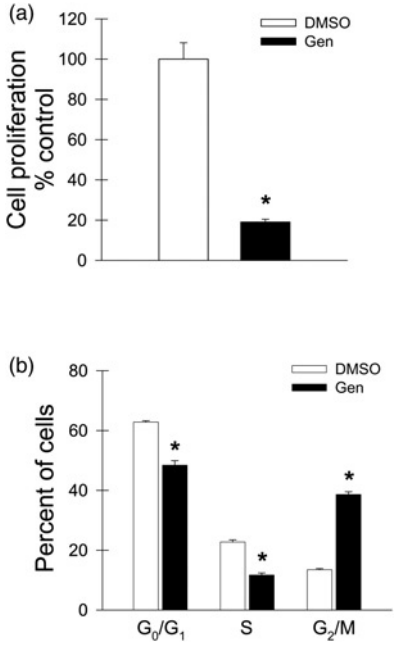

Figure 1 Genistein impairs lung cancer cell proliferation by blocking cell cycle progression. H460 lung cancer cells were treated with 100 μmol/L genistein (Gen) or DMSO (D) for 48 h and cell proliferation was determined by crystal violet assay (a). In cells undergoing similar genistein treatment, the distribution of cells in cell cycle phases was determined by fluorocytometry (b). $^*P < 0.05$ or less versus vehicle-treated cells, by Student's *t*-test. DMSO, dimethyl sulfoxide

Genistein reduces SCD1 protein levels and activity in cancer cells

Previous studies have shown that SCD1 is overactivated in cancer cells and plays a critical role in supporting the biochemical and biological phenotype of cancer cells.[5,22–25] Since SCD1 expression and activity are modulated by a large number of hormones and dietary factors, isoflavones may target SCD1 for modulation in H460 cells. As shown in Figure 2a, treatment with 100 μmol/L genistein

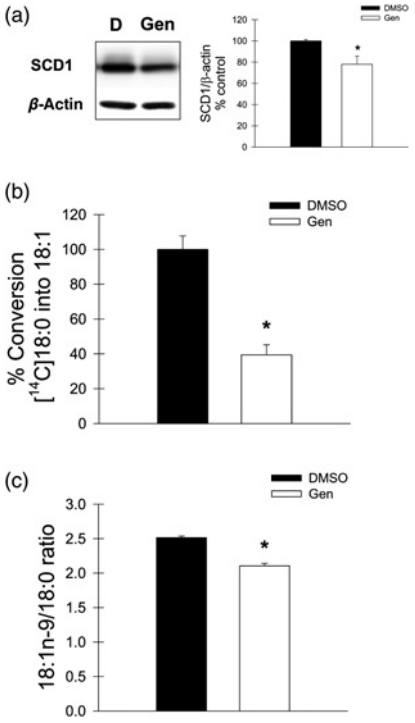

Figure 2 Genistein decreases SCD1 levels and MUFA/SFA ratio in total lipids of lung cancer cells. In H460 cells treated with 100 μmol/L genistein (Gen) or DMSO for 48 h, the levels of SCD1 and control β-actin were determined by Western blot (a). For the determination of Δ9-desaturating activity (b), cells were incubated with 100 μmol/L genistein (Gen) or DMSO for 48 h. Six hours before harvesting, the cells were pulsed with [^{14}C]18:0 (0.25 μCi/dish). After conversion into methylesters, SFA and MUFA were resolved on silver nitrate-impregnated thin-layer chromatography plates. The radioactive spots corresponding to [^{14}C]18:0 substrate and product ([^{14}C]18:1) were visualized with a Phosphor Imager and quantified by densitometric analysis. (c) Ratio 18:1n-9/18:0 in total lipids was determined in cells under similar genistein treatment conditions. To do so, cellular lipids were extracted and fatty acids were converted to their methylester form by transesterification as described in Materials and methods. Fatty acid methyl ester composition was assessed by gas chromatography and percent distribution of fatty acids was calculated. Values represent the mean ± SD of triplicate determinations. $^*P < 0.05$ versus vehicle-treated controls, by Student's *t*-test. MUFA, monounsaturated fatty acid; SFA, saturated fatty acid; DMSO, dimethyl sulfoxide; SCD1, stearoyl-CoA desaturase-1

decreased SCD1 protein levels in H460 cancer cells. In order to assess whether the reduction of SCD1 levels by genistein affected the overall rate of SCD activity, we determined the conversion of [^{14}C]stearic acid into oleic acid in H460 cells treated with 100 μmol/L genistein. Indeed, incubation with genistein led to a marked reduction in the production of oleic acid in cancer cells (Figure 2b). Further, inhibition of SCD1 by genistein produced a ~25% decrease in the ratio of 18:1n-9/18:0, two of the most abundant fatty acids in cancer cells, indicating that by downregulating SCD1, the isoflavone alters the fatty acid profile of these cells.

Genistein suppresses *de novo* synthesis of lipids

Cancer cells require an active production of SFA and MUFA to fulfill their requirements for lipid structures, metabolic fuel and signals appropriate for rapid proliferation.[2–4] Having observed that genistein targets SCD1 activity for inhibition, we next investigated the effects of genistein on overall lipid biosynthesis using [^{14}C]acetate as a tracer. Upon conversion into acetyl-CoA by acetyl-CoA synthetase, acetate enters the routes of fatty acid biosynthesis and cholesterol synthesis. As shown in Figure 3, radiolabeled acetate was preferentially incorporated into phospholipids of cancer cells (~75%). The remaining [^{14}C]tracer incorporated into triacylglycerol (TAG) pools (18–20%) and cholesterolesterase (CE) (~5%) (Figure 3a). Among phospholipids, incorporation of [^{14}C]acetate was greater in phosphatidylcholine, with ~50% of total acetate labeling, followed by phosphatidylethanolamine (~13%) (Figure 3b), phosphatidylinositol (~6%), phosphatidylserine (~3%) and lysophosphatidylcholine (1.5%) (Figure 3c). The overall synthesis of lipids from acetate was markedly reduced by genistein. Levels of [^{14}C]acetate were reduced between 40% and 70% in phospholipid and neutral lipid species (Figures 3a–c). Altogether, these findings suggest a global downregulating effect of genistein on *de novo* lipid synthesis, likely as the result of a reduction in the fatty acid biosynthetic pathway.

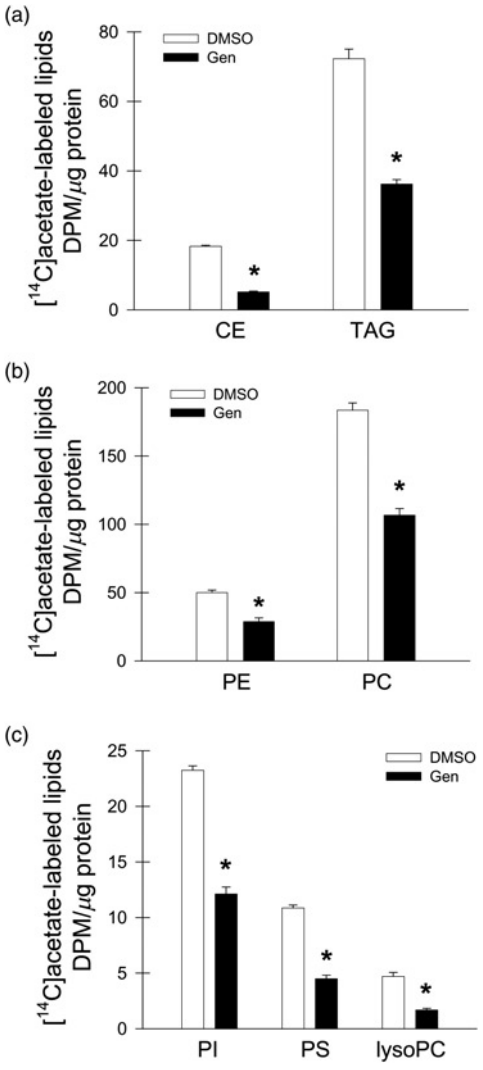

Figure 3 *De novo* lipid synthesis in lung cancer cells is inhibited by genistein. The rate of *de novo* lipid synthesis was determined in H460 cells incubated with 100 μmol/L genistein (Gen) or DMSO for 48 h. During the last 24 h of incubation, cells were radiolabeled with 0.5 μCi/dish of [^{14}C]acetate. Lipids were extracted and polar and neutral species were separated by one-dimensional thin-layer chromatography. Radioactive lipid spots were identified in a Phosphor Imager and quantified by densitometric analysis. (a) CE, cholesterolesteres, TAG, triacylglycerols; (b) PE, phosphatidylethanolamine, PC, phosphatidylcholine; (c) PI, phosphatidylinositol; PS, phosphatidylserine; LysoPC, lysophosphatidylcholine. Values represent the mean ± SD of triplicate determinations. $^{*}P < 0.05$ or less versus vehicle-treated controls, by Student's *t*-test. DMSO, dimethyl sulfoxide; DPM, disintegrations per minute

Genistein increases the incorporation of exogenous fatty acids into cellular lipid pools

Cancer cells rely almost exclusively on endogenously synthesized fatty acids for the production of new lipid macromolecules.[3,4] Since genistein induced a dramatic decrease in *de novo* lipid biosynthesis in cancer cells, we wished to investigate if the incorporation of exogenous fatty acids into lipid pools was affected by genistein. Thus, genistein- and vehicle-treated cancer cells were incubated with [^{14}C]stearic acid for six hours, and levels of the major radiolabeled lipids were determined (Figure 4). In neutral lipid fractions, [^{14}C]fatty acid was mainly acylated into TAG and CE (Figure 4a), with minor labeling in unesterified fatty acid and diacylglycerol fractions (data not shown). Similar to cells traced with radiolabeled acetate, the largest fraction of exogenous [^{14}C]fatty acid (~90%) was incorporated into the phospholipid pool (Figures 4b and c), in which phosphatidylcholine (PC) and phosphatidylethanolamine fractions were the main labeled polar lipid species (Figure 4b) followed by phosphatidylinositol, phosphatidylserine and lysophosphatidylcholine (Figure 4c). Treatment with 100 μmol/L genistein promoted a global increase in the acylation of main lipid fractions with [^{14}C]fatty acid, although the increase in [^{14}C]fatty acid-labeled TAG was proportionally greater that the other lipid macromolecules (Figure 4a). These observations suggest the presence of a compensatory mechanism in cells treated with the isoflavone that overcomes the low production of endogenous lipids by increasing the uptake of exogenous fatty acids and their incorporation into different lipid species. Moreover, our findings that the level of TAG formation from exogenous [^{14}C]stearic acid is inversely correlated to the degree of SCD1 activity suggest that TAG is the preferential lipid pool for the storage of SFA.

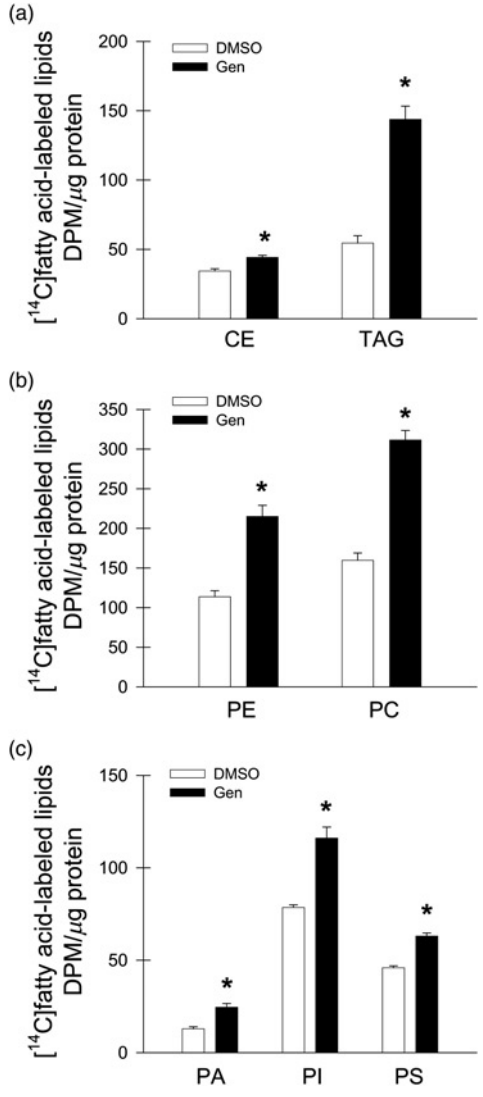

Figure 4 [^{14}C]Stearic acid incorporates preferentially into TAG of genistein-treated cells. H460 cells were grown until 80–90% confluent and treated with 100 μmol/L genistein (Gen) or DMSO in 10% fetal bovine serum Dulbecco's modified Eagle's medium for 48 h. Cells were incubated with [^{14}C]stearic acid for the last six hours of treatment, and cell lipids were extracted and different species were separated by thin-layer chromatography. Radioactive lipid spots were detected with a Phosphor Imager and quantified by densitometry. (a) CE, cholesterolesteres; TAG, triacylglycerols. (b) PE, phosphatidylethanolamine; PC, phosphatidylcholine.(c) PI, phosphatidylinositol; PS, phosphatidylserine; PA, phosphatidic acid. Values represent the mean $\pm$ SD of triplicate determinations. $^{*}P < 0.05$ or less versus vehicle-treated controls, by Student's *t*-test. DPM, disintegrations per minute

Discussion

Aberrant expression of enzymes of lipogenesis, particularly fatty acid biosynthetic enzymes, has been linked to the onset and progression of most prevalent metabolic diseases such as obesity, diabetes and cardiovascular disease.[26,27] Furthermore, an abnormally high rate of *de novo* lipid synthesis has been associated with the pathogenesis of cancer.[4] In cancer cells and animal tumor models, the pharmacological and genetic ablation of ACL, ACC, fatty acid synthase (FAS) and SCD1 promoted a downregulation of lipid biosynthesis as well as attenuation of malignant phenotype.[10–25] Therefore, lipogenic enzymes are increasingly becoming valuable targets for nutritional and pharmacological interventions in cancer therapy. Our studies demonstrate that genistein, a prototypical nutraceutical compound, promotes a global downregulation of *de novo* lipid synthesis and a marked decrease in the proliferation rate of lung cancer cells. With regard to lipogenesis, a reduction in lipid biosynthesis by genistein was reported in isolated rat adipocytes,[12] suggesting that this phytoestrogen is able to modulate this metabolic pathway in highly lipogenic cells. Our observation of an overall reduction in *de novo* lipid biosynthesis in lung cancer cells by genistein indicates that this isoflavone may affect central regulatory mechanisms for the control of lipogenesis. The suppression of lipid biosynthesis by genistein may be caused by targeting SREBP-1, a key transcriptional factor that controls critical enzymes involved in the biosynthesis of fatty acids and triacylglycerols such as glycerol-3-phosphate acyltransferase (GPAT), FAS and SCD1.[28] In this connection, previous studies have shown that isoflavones are able to suppress the activation of SREBP-1.[29]

The observed antilipogenic effect of genistein in lung cancer cells may be produced, at least in part, by targeting the MUFA synthesis. We detected a significant reduction of SCD1 protein levels in genistein-treated cells, in agreement with similar observations reported by Shin *et al.*[16] and Su *et al.*[29] in HepG2 cells and differentiated adipocytes, respectively. The marked decrease in SCD activity and, more importantly, a significant reduction in the ratio oleic acid to stearic acid observed in cancer cells treated with genistein was likely caused by the lower expression of SCD1 protein. The potential implications of the perturbation in the fatty acid distribution promoted by genistein are vast since changing ratios of MUFA to SFA modify the physicochemical properties of cell membranes, with a consequent impact on the activity of membrane-resident proteins, such as enzymes, transporter and cytokine receptors. An altered lipid composition may hinder the activation of tyrosine-kinase receptors such as insulin, PDGF, EGF, fibroblast growth factor (FGF) and keratinocyte growth factor (KGF), which are known to stimulate lipid synthesis and mitogenesis in human cells.[30–32] Genistein may also suppress lipogenic activity in cancer cells by directly blocking the tyrosine kinase activity of EGF receptors.[33] Furthermore, several biological activities of isoflavones are due to their capacity to bind and activate estrogen receptors.[12] However, it is unlikely that genistein impairs lipid formation through its estrogen-like effects since estrogen has been shown to increase MUFA levels in chicken liver by inducing SCD1 expression.[34]

Besides affecting the production of new lipids, genistein also produced notable changes in the segregation of fatty acids into cell lipid pools. The greater incorporation of exogenous stearic acid into all major lipids in cancer cells treated with the phytoestrogen suggests a depletion of endogenously synthesized fatty acids. This finding also indicates that the acylation of fatty acids into glycerolipids and cholesterolesters is not inhibited by genistein, further adding evidence to the notion that genistein specifically targets the biosynthesis and desaturation of fatty acids in cancer cells. Moreover, although the acylation of exogenous fatty acids was increased in both membrane lipids and storage lipids, the fact that stearic acid preferentially

segregated into TAG may point out to a safeguard mechanism for sequestering the excess of SFA in genistein-treated cells. The downregulation of SCD1 promoted by genistein may force the partition of SFA into TAG since a similar segregation of SFA away from phospholipids and into neutral lipids was detected in SCD1-ablated cancer cells.[6] Accumulation of SFA are know to promote cell stress and, ultimately, to trigger apoptosis[35–37] and the presence of active SCD1 in cancer cells prevents this lipotoxic phenomenon by converting harmful SFA into less deleterious MUFA.[4] It is then conceivable that the antigrowth action of genistein in cancer cells could be, at least partly, attributed to a toxic accumulation of SFA due to SCD1 depletion.

As previously reported by Lian *et al.*,[38] genistein treatment led to cell cycle arrest in H460 cells by inducing a blockade in the G_2/M phase, which explains the observed cessation of cell proliferation. Arrest of cancer cell cycle in the G_2/M phase induced by isoflavones has been reported in a number of cancer cells including breast, prostate and lung cancer.[21] We also detected a decrease in the progression of cell cycle from the G_0/G_1 to the S phase, which can be attributed to the use of a greater genistein concentration than that used in the aforementioned studies. Interestingly, the reduction of SCD1 in genistein-treated cells may have also induced a similar block in cell cycle since the pharmacological blockade of SCD1 activity was shown to induce arrest of H460 cancer cells in the G_0/G_1 phase.[9] A reduction in the population of cells in these cell cycle segments has also been detected in melanoma cells and mouse fibroblasts.[19] Isoflavones may affect the rate of cell replication by modifying lipid homeostasis since active lipogenesis is essential for the progression of cell cycle in cancer cells and for avoiding the entry in the cell death program.[39] We and others have shown that the ablation of the fatty acid biosynthetic enzymes ACC, FAS and SCD1 blocks the progression of cell cycle in the G_0/G_1 phase.[9,10,22–25] Nevertheless, although a perturbation in the fatty acid biosynthetic program by genistein may contribute to a blockade of cell cycle progression through the $G_1 \rightarrow S$ boundary, it cannot explain the blockade of G_2/M phases commonly seen in cancer cells undergoing treatment with isoflavone compounds. In this regard, genistein is known to impair the activation of the cdc-2/cyclin B complex, a critical event in the G_2/M procession.[20]

In summary, we have characterized the effect of genistein on lipid biosynthesis in a model of lung cancer cells. We observed that the *de novo* formation of major phospholipid and neutral lipid species is dramatically reduced by genistein, likely by targeting fatty acid synthesis and desaturation. In this regard, genistein exhibits a strong downregulating effect on SCD1, a key lipogenic enzyme that controls lung cancer cell proliferation, survival and tumorigenesis. Along with these significant perturbations in lipid biosynthesis, genistein markedly reduced proliferation of lung cancer cells by arresting the cell cycle. The fact that abnormally high activity levels of the fatty acid synthetic pathway has been associated with the onset and progression of cancer provides evidence in support of a potential therapeutic use of genistein in malignant diseases, particularly lung cancer, a lethal form of cancer for which effective treatment is lacking.

Authors contributions: RAI conceived and designed the experiments; DH and RAI performed the experiments, analyzed the data and wrote the paper.

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REFERENCES

1 Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin* 2010;**60**:277–300
2 DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab* 2008;**7**:11–20
3 Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nat Rev Cancer* 2007;**7**:763–77
4 Igal RA. Stearoyl-CoA desaturase-1: a novel key player in the mechanisms of cell proliferation, programmed cell death and transformation to cancer. *Carcinogenesis* 2010;**31**:1509–15
5 Scaglia N, Caviglia JM, Igal RA. High stearoyl-CoA desaturase protein and activity levels in simian virus 40 transformed-human lung fibroblasts. *Biochim Biophys Acta* 2005;**1687**:141–51
6 Scaglia N, Igal RA. Stearoyl-CoA desaturase is involved in the control of proliferation, anchorage-independent growth, and survival in human transformed cells. *J Biol Chem* 2005;**280**:25339–49
7 Scaglia N, Igal RA. Inhibition of stearoyl-CoA desaturase 1 expression in human lung adenocarcinoma cells impairs tumorigenesis. *Int J Oncol* 2008;**33**:839–50
8 Morgan-Lappe SE, Tucker LA, Huang X, Zhang Q, Sarthy AV, Zakula D, Vernetti L, Schurdak M, Wang J, Fesik SW. Identification of Ras-related nuclear protein, targeting protein for xenopus kinesin-like protein 2, and stearoyl-CoA desaturase 1 as promising cancer targets from an RNAi-based screen. *Cancer Res* 2007;**67**:4390–8
9 Hess D, Chisholm JW, Igal RA. Inhibition of stearoyl CoA desaturase activity blocks cell cycle progression and induces programmed cell death in lung cancer cells. *PLoS One* 2010;**5**:e11394
10 Scaglia N, Chisholm JW, Igal RA. Inhibition of stearoyl-CoA desaturase 1 inactivates acetyl-CoA carboxylase and impairs proliferation in human cancer cells. Role of AMPK. *PLoS One* 2009;**4**:e6812
11 Schmidt BM, Ribnicky DM, Lipsky PE, Raskin I. Revisiting the ancient concept of botanical therapeutics. *Nat Chem Biol* 2007;**3**:360–6
12 Szkudelska K, Nogowski L. Genistein – a dietary compound inducing hormonal and metabolic changes. *J Steroid Biochem Mol Biol* 2007;**105**:37–45
13 Price KR, Fenwick GR. Naturally occurring oestrogens in foods – a review. *Food Addit Contam* 1985;**2**:73–106
14 El-Zarruk AA, van den Berg HW. The anti-proliferative effects of tyrosine kinase inhibitors towards tamoxifen-sensitive and tamoxifen-resistant human breast cancer cell lines in relation to the expression of epidermal growth factor receptors (EGF-R) and the inhibition of EGF-R tyrosine kinase. *Cancer Lett* 1999;**142**:185–93
15 Szkudelska K, Nogowski L, Szkudelski T. Genistein affects lipogenesis and lipolysis in isolated rat adipocytes. *J Steroid Biochem Mol Biol* 2000;**75**:265–71
16 Shin ES, Lee HH, Cho SY, Park HW, Lee SJ, Lee TR. Genistein downregulates SREBP-1 regulated gene expression by inhibiting site-1 protease expression in HepG2 cells. *J Nutr* 2007;**137**:1127–31
17 Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;**37**:911–7
18 Wilson R, Sargent JR. Chain separation of monounsaturated fatty acid methyl esters by argentation thin-layer chromatography. *J Chromatogr A* 2001;**905**:251–7

19 Kuzumaki T, Kobayashi T, Ishikawa K. Genistein induces p21(Cip1/WAF1) expression and blocks the G1 to S phase transition in mouse fibroblast and melanoma cells. *Biochem Biophys Res Commun* 1998;**251**:291–5
20 Touny LH, Banerjee PP. Identification of both Myt-1 and Wee-1 as necessary mediators of the p21-independent inactivation of the cdc-2/cyclin B1 complex and growth inhibition of TRAMP cancer cells by genistein. *Prostate* 2006;**66**:1542–55
21 Banerjee S, Li Y, Wang Z, Sarkar FH. Multi-targeted therapy of cancer by genistein. *Cancer Lett* 2008;**269**:226–42
22 Chajes V, Cambot M, Moreau K, Lenoir GM, Joulin V. Acetyl-CoA carboxylase alpha is essential to breast cancer cell survival. *Cancer Res* 2006;**66**:5287–94
23 Kuhajda FP, Jenner K, Wood FD, Hennigar RA, Jacobs LB, Dick JD, Pasternack GR. Fatty acid synthesis: a potential selective target for antineoplastic therapy. *Proc Natl Acad Sci USA* 1994;**91**:6379–83
24 Beckers A, Organe S, Timmermans L, Scheys K, Peeters A, Brusselmans K, Verhoeven G, Swinnen JV. Chemical inhibition of acetyl-CoA carboxylase induces growth arrest and cytotoxicity selectively in cancer cells. *Cancer Res* 2007;**67**:8180–7
25 Hatzivassiliou G, Zhao F, Bauer DE, Andreadis C, Shaw AN, Dhanak D, Hingorani SR, Tuveson DA, Thompson CB. ATP citrate lyase inhibition can suppress tumor cell growth. *Cancer Cell* 2005;**8**:311–21
26 Ntambi JM, Miyazaki M. Regulation of stearoyl-CoA desaturases and role in metabolism. *Prog Lipid Res* 2004;**43**:91–104
27 Brown JM, Rudel LL. Stearoyl-coenzyme A desaturase 1 inhibition and the metabolic syndrome: considerations for future drug discovery. *Curr Opin Lipidol* 2010;**21**:192–7
28 Goldstein JL, DeBose-Boyd RA, Brown MS. Protein sensors for membrane sterols. *Cell* 2006;**124**:35–46
29 Su Y, Shankar K, Simmen RC. Early soy exposure via maternal diet regulates rat mammary epithelial differentiation by paracrine signaling from stromal adipocytes. *J Nutr* 2009;**139**:945–51
30 Samuel W, Nagineni CN, Kutty RK, Parks WT, Gordon JS, Prouty SM, Hooks JJ, Wiggert B. Transforming growth factor-beta regulates stearoyl coenzyme A desaturase expression through a Smad signaling pathway. *J Biol Chem* 2002;**277**:59–66
31 Demoulin JB, Ericsson J, Kallin A, Rorsman C, Ronnstrand L, Heldin CH. Platelet-derived growth factor stimulates membrane lipid synthesis through activation of phosphatidylinositol 3-kinase and sterol regulatory element-binding proteins. *J Biol Chem* 2004;**279**:35392–402
32 Chang Y, Wang J, Lu X, Thewke DP, Mason RJ. KGF induces lipogenic genes through a PI3K and JNK/SREBP-1 pathway in H292 cells. *J Lipid Res* 2005;**46**:2624–35
33 Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M, Fukami Y. Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem* 1987;**262**:5592–5
34 Hermier D, Catheline D, Legrand P. Relationship between hepatic fatty acid desaturation and lipid secretion in the estrogenized chicken. *Comp Biochem Physiol A Physiol* 1996;**115**:259–64
35 de Vries JE, Vork MM, Roemen TH, de Jong YF, Cleutjens JP, van der Vusse GJ, van Bilsen M. Saturated but not mono-unsaturated fatty acids induce apoptotic cell death in neonatal rat ventricular myocytes. *J Lipid Res* 1997;**38**:1384–94
36 Schaffer JE. Lipotoxicity: when tissues overeat. *Curr Opin Lipidol* 2003;**14**:281–7
37 Wei Y, Wang D, Topczewski F, Pagliassotti MJ. Saturated fatty acids induce endoplasmic reticulum stress and apoptosis independently of ceramide in liver cells. *Am J Physiol Endocrinol Metab* 2006;**291**:E275–81
38 Lian F, Bhuiyan M, Li YW, Wall N, Kraut M, Sarkar FH. Genistein-induced G_2–M arrest, p21WAF1 upregulation, and apoptosis in a non-small-cell lung cancer cell line. *Nutr Cancer* 1998;**31**:184–91
39 Cui Z, Houweling M, Chen MH, Record M, Chap H, Vance DE, Terce FA. Genetic defect in phosphatidylcholine biosynthesis triggers apoptosis in Chinese hamster ovary cells. *J Biol Chem* 1996;**271**:14668–71

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