Green Tea Catechin Inhibits Fatty Acid Synthase without Stimulating Carnitine Palmitoyltransferase-1 or Inducing Weight Loss in Experimental Animals

TERESA PUIG[1,2*], JOANA RELAT[3*], PEDRO F. MARRERO[3], DIEGO HARO[3], JOAN BRUNET[1] and RAMON COLOMER[1,4]

[1]*Girona Biomedical Research Institute (IdIBGi) and Catalan Institute of Oncology, Dr. Josep Trueta University Hospital, Girona;*
[2]*Biochemistry and Molecular Biology, School of Biology, University of Girona, Girona;*
[3]*Biochemistry and Molecular Biology, School of Pharmacy, University of Barcelona, Barcelona;*
[4]*MD Anderson Cancer Center España, Madrid, Spain*

Abstract. *Background: The enzyme fatty acid synthase (FASN) is highly expressed in many human carcinomas and its inhibition is cytotoxic to human cancer cells. The use of FASN inhibitors has been limited until now by anorexia and weight loss, which is associated with the stimulation of fatty acid oxidation. Materials and Methods: The in vitro effect of (–)-epigallocatechin-3-gallate (EGCG) on fatty acid metabolism enzymes, on apoptosis and on cell signalling was evaluated. In vivo, the effect of EGCG on animal body weight was addressed. Results: EGCG inhibited FASN activity, induced apoptosis and caused a marked decrease of human epidermal growth factor receptor 2 (HER2), phosphatidylinositol 3-kinase (PI3K)/AKT and extracellular (signal)-regulated kinase (ERK) 1/2 proteins, in breast cancer cells. EGCG did not induce a stimulatory effect on CPT-1 activity in vitro (84% of control), or on animal body weight in vivo (99% of control). Conclusion: EGCG is a FASN inhibitor with anticancer activity which does not exhibit cross-activation of fatty acid oxidation and does not induce weight loss, suggesting its potential use as an anticancer drug.*

Mammalian fatty acid synthase (FASN; EC 2.3.1.85) is a complex multifunctional enzyme that catalyzes the synthesis of palmitate from substrates acetyl-CoA, malonyl-CoA and nicotinamide adenine dinucleotide phosphate (NADPH) (1). The endogenous synthesis of fatty acids is usually minimal in animals because the diet supplies most of the fatty acids and, consequently, FASN is expressed at low or undetectable levels in non-malignant cells. In contrast, high levels of FASN have been reported in breast cancer and other human solid carcinomas (2-7). Treatment of cancer cells with some pharmacological inhibitors of FASN, such as the natural product cerulenin and its synthetic derivative C75 (8), are cytotoxic to breast cancer cells both *in vitro* (8-12) and *in vivo* (13). These FASN inhibitors provided the first evidence of anticancer activity, although they induced a profound decrease in food intake and body weight in rodents (14). C75 also activates different components of the carnitine palmitoyltransferase (CPT) system (15-17). The CPT system controls the entry of the long-chain fatty acids into the mitochondria, where they undergo β-oxidation. CPT-1 catalyses the first rate-limiting step in the CPT shuttle system and it is considered the most critical step in controlling fatty acid flux through its physiological inhibition by malonyl-CoA (15-18). Interestingly, CPT-1 stimulation appears important for the C75 anorexic effect, since selective CPT-1 pharmacological activation induces animal weight loss (19).

The biologically active anticancer components of green tea include polyphenolic catechins (20, 21). Several studies have indicated that epigallocatechin-3-gallate (EGCG) is the most abundant and biologically active catechin with respect to anticancer activity (22, 23). Different potential mechanisms contributing to the anticancer effects of tea catechin EGCG have been described, including blockade of methylation, inhibition of metalloproteinase and receptor tyrosine kinases (reviewed in 24). Recently, we and others have reported that EGCG induced apoptosis in cancer cells through the inhibition of FASN activity (12, 22, 25-29).

The aim of the present study was to search for a specific FASN inhibitor devoid of weight loss effects *in vivo*. For the

*Both authors contributed equally to this study.

Correspondence to: Teresa Puig Miquel, Ph.D., Cancer Drug Development, Catalan Institute of Oncology and Girona Biomedical Research Institute (IdIBGi), Avda. França s/n; E-17007 Girona, Spain. Tel: +34 972940282, Fax: +34 972485422, e-mail: mtpuig@iconcologia.es

Key Words: Breast cancer, green tea catechins, fatty acid synthase, animal model, weight loss, anti-cancer drug.

first time to our knowledge, the effect of EGCG on CPT-1 activity *in vitro* and on body weight *in vivo* was evaluated. Additionally, evidence for the molecular signaling pathways involved in the cytotoxic effects was determined.

Materials and Methods

Cell lines and reagents. SK-Br3 was used as an optimal human breast cancer cell line due to its high constitutive FASN and human epidermal growth factor receptor 2 (HER2) expression and activity levels. The SK-Br3 cells were purchased from Eucellbank (Barcelona, Spain) and were cultured in McCoy's 5A medium (Gibco, Berlin, Germany) containing 10% foetal bovine serum (FBS; Bio-Whittaker, Walkersville, MD, USA) 1% L-glutamine, 1% sodium pyruvate, 50 U/ml penicillin and 50 μg/ml streptomycin. EGCG and C75 were obtained from Sigma (St. Louis, MO, USA). The primary antibody for FASN immunoblotting was a mouse IgG1 FAS monoclonal antibody obtained from BD Biosciences Pharmingen (San Diego, CA, USA). Monoclonal anti-β-actin mouse antibody (clone AC-15) was obtained from Sigma. Anti-AKT, anti-phospo-AKT^{Ser473} rabbit polyclonal antibodies and mouse monoclonal antibodies against p185$^{HER-2/neu}$ (clone Ab-3) and phospo-p185$^{HER-2/neu}$ were purchased from Cell Signaling Technology (Beverly, MD, USA). The assay of CPT-1 activity was conducted using palmitoyl-CoA lithium salt from Sigma, fatty acid-free BSA from Roche (Manheim, Germany), L-carnitine hydrochloride from Sigma and L-[methyl-^{3}H] carnitine hydrochloride (82Ci/mmol) from Amersham Biosciences (Piscataway, NJ, USA).

Mouse model. Twelve-week-old C57BL/6J male mice were purchased from Harlan Laboratories (Gannat, France). The mice were fed *ad libitum* with a standard rodent chow through out the experimental procedures. Mice were maintained in a 12 h light-dark cycle at 22˚C. After a 1-week acclimatization, the animals were treated as described.

Fatty acid synthase activity assay. The cells were harvested by treatment with trypsin-EDTA solution, pelleted by centrifugation, washed twice and resuspended in ice-cold PBS. The cells were sonicated for 30 min at 4˚C (P-Selecta ultrasons, Barcelona, Spain) and centrifuged for 15 min at 4˚C to keep the supernatants particle-free. A sample was taken to measure the protein content measured by the Lowry-based BioRad assay (BioRad Laboratories, Hercules, CA, USA). FASN activity, expressed in nmol NADPH oxidized / min × mg protein, was assayed in the particle-free supernatant samples of equal protein content by spectrophotometrically recording the decrease of $A_{340\ nm}$ due to oxidation of NADPH at 37˚C (LambdaBio 20, PerkinElmer, MA, USA) using UV Kinlab 2.80.02 software (PerkinElmer) as previously described (12).

Quantitative analysis of apoptotic cells by flow cytometry. The quantitative analysis of apoptotic cell death caused by the EGCG treatment was conducted by flow cytometry using the Annexin V-Alexa Fluor 488 Apoptosis Detection Kit (Molecular Probes, Eugene, OR, USA) following the manufacturer's instructions. Briefly, after treatment with EGCG for 12, 24 or 48 h, the SK-Br3 cells were harvested, washed in cold PBS and subjected to Annexin V-Alexa Fluor 488 (Alexa488) and propidium iodide (PI) staining in binding buffer at room temperature for 10 min in the dark. The stained cells were analyzed by fluorescence-activated cell sorting (FACSCalibur; BD Biosciences, San Jose, CA, USA) using CellQuest 3.3 software (BD Biosciences).

Immunoblot analysis of FASN, p185$^{HER2/neu}$, phospo-p185$^{HER2/neu}$, anti-ERK1/2, anti-phospo-ERK1/2, anti-AKT and anti-phospo-AKT^{Ser473}. Overnight serum-starved SK-Br3 cells were treated with 150 μM EGCG for the desired time intervals. The cells were scraped with trypsin-EDTA solution, washed twice with ice-cold PBS and homogenized in lysis buffer (1 mM EDTA, 150 mM NaCl, 100 μg/ml α–toluene sulphonyl fluoride (PMSF) and 50 mM Tris-HCl, pH 7.5). A sample was taken for measurement of protein content by the Lowry-based BioRad assay. Equal amounts of protein were heated in sodium dodecyl sulphate (SDS) sample buffer for 5 min at 95˚C, separated on a 3-8% SDS-polyacrylamide gel (FASN, p185$^{HER2/neu}$, phospo-p185$^{HER2/neu}$) or 4-12% SDS-polyacrylamide gel (AKT, phospho-AKT, extracellular (signal)-regulated kinase (ERK)1/2 and phospo-ERK1/2) and transferred onto nitrocellulose membranes. The membranes were incubated for 1 h at room temperature in blocking buffer (2.5% powdered-skim milk in TBS-T [10 mM Tris-CIH pH 8.0, 150 mM NaCl and 0.05% Tween-20]) to prevent non-specific antibody binding, and incubated with the corresponding primary antibody diluted in blocking buffer overnight at 4˚C. After 3×5 min washing in TBS-T, blots were incubated for 1 h with anti-mouse IgG peroxidase conjugate and revealed employing a commercial kit (West Pico chemiluminescent substrate; Pierce Biotechnology, Rockford, USA). The blots were re-probed with an antibody for β-actin to control for protein loading and transfer.

Measurement of carnitine palmitoyltransferase-1 (CPT-1) activity. CPT-1 activity was assayed by the forward exchange method using L-[^{3}H]carnitine as previously described (12, 16) Briefly, the reaction mixture (total volume of 0.5 ml) consisted of the standard enzyme assay mixture with 200 μM C75 or EGCG, 0.2 mM L-[^{3}H]carnitine (~5000 dpm/nmol), 80 μM palmitoyl-CoA, 20 mM HEPES (pH 7.0), 1% fatty acid-free albumin and 40-75 mM KCl, with or without malonyl-CoA (10 μM) as indicated. The reactions were initiated by the addition of isolated intact yeast mitochondria expressing recombinant CPT1A (16). The reaction was linear up to 4 min, and all the incubations were conducted at 30˚C for 3 min. The reactions were stopped by the addition of 6% perchloric acid and the samples were then centrifuged at 2300 rpm for 5 min. The resulting pellet was suspended in water and the product [^{3}H] palmitoylcarnitine was extracted with butanol at low pH. After centrifugation at 2500 rpm for 3 min, an aliquot of the butanol phase was transferred to a vial for radioactive counting.

Animal preparation for in vivo experiments. The animals were randomized into three groups of 5 animals each: control, C75-treated and EGCG-treated. All the experiments were conducted in accordance with guidelines on animal care and use established by the University of Barcelona School of Farmacia institutional animal care and scientific committee. The treatments were conducted as previously described by Cha *et al*. (30). Briefly, the mice were weighed (Fed) for 12 h during the dark cycle and weighed (Fasted) before treatment. Each group received a single intraperitoneal (*i.p.*) injection (0.5 ml) of FASN inhibitor (30 mg/kg) or vehicle alone (DMSO), disolved in RPMI-1640 medium (Invitrogen, Life Technologies, Carslbad, CA, USA). After the *i.p.* injections, the animals were given free access to rodent chow for 24 h, at which time, the experiment finished and the animals were weighed again (Refed).

Statistical analysis. The data from the *in vitro* and *in vivo* results were analyzed by Student's *t*-test or by one-way ANOVA using a Tukey test as a post-test. The data are reported as means±SD. All the observations were confirmed by at least three independent experiments. For all the tests, $p<0.05$ was considered statistically significant.

Results

Effect of EGCG on FASN and CPT-1 activity. Highlighted in Table I are the activity values of EGCG compared with C75 (included for comparative purposes) for key fatty acid metabolism enzymes. The inhibitor concentrations used were the IC_{50} values of EGCG (150 μM) and C75 (30 μM) against SKBr-3 human breast cancer cells (data not shown). EGCG markedly reduced FASN activity in the SKBr-3 cells compared to the control (59±13%), comparable to the reduction obtained with C75 (43±4%). EGCG had no effect on the abundance of FASN protein levels, which was measured from the same treated samples by Western blot. EGCG did not exert any significant effect on CPT-1 activity (84±16%, respect to control), in sharp contrast to C75 which, as previously reported, produced substantial activation of CPT-1 both in the absence (30.0%, in respect to control) and in the presence of inhibitory concentrations of malonyl-CoA (28.8%, in respect to control), a physiological inhibitor of CPT-1 (data not shown).

Effect of EGCG on apoptosis and oncogene HER2 activation. The treatment of the cancer cells with EGCG time-dependently increased the percentage of apoptotic cells, the induction of apoptosis was higher when the cells were treated with EGCG for 24 or 48 h (Figure 1A). The number of late apoptotic cells increased from 0.6% in non-EGCG treated cells to 11.2% with EGCG treatment for 24 h. Thus, the total percentage of apoptotic cells (UR + LR) increased from 1% in the control cells to 16.2% following the treatment with EGCG for 24 h (Figure 1A; panels C and D). Similarly, treatment of the SK-Br3 cells with EGCG for 48 h further increased the apoptosis. The number of late apoptotic cells increased from 0.7% in the non-treated cells to 21.2% in the cells treated with EGCG for 48 h. Thus, the total percentage of apoptotic cells (UR + LR) increased from 1.2% in the non-treated cells to 27.5% following the 48 h treatment with EGCG 150 μM (Figure 1A; panels E and F). Apoptosis and the induction of caspase activity was also confirmed by Western blot analysis showing cleavage of poly(ADP-ribose) polymerase (PARP) after EGCG treatment (data not shown).

Figure 1B shows that EGCG dramatically reduced HER2 phosphorylation (p-HER2) within 6 h after treatment; reduction was already marked as soon as 2 h after EGCG treatment (data not shown) and was completed by 6 h after treatment. During this period, there was no significant change in the total level of HER2, as assessed by Western blotting analysis (Figure 1B) or by HER2-specific ELISA (data not shown). Similarly, EGCG markedly reduced the expression levels of both, p-AKT and p-ERK1/2 proteins within 12 h after exposure. During this period, there was no significant change in the total level of the respective proteins (Figure 1B). Together, these changes were not due to a reduction in FASN protein levels, as confirmed by immunoblot analysis, using the same treated samples in which FASN activity was measured (Figure 1B).

Table I. *Effect of green tea catechin on fatty acid metabolism pathways*

Compound	FASN inhibition[a]	CPT-1 stimulation[b]
EGCG	58.9±12.8†	84±16
C75	43.3±4.1†	129±18*
Vehicle (control)	N.E.	N.E.

FASN: Fatty acid synthase; CPT-1: carnitine palmitoyltransferase-1; N.E.: No effect; [a]Data are % nmol of NADPH oxidized/min × mg protein of control cells; [b]Data are % mU/mg × min of control cells; Data are expressed as mean±SD (N=3). *$p<0.05$, †$p<0.01$.

Weight loss in vivo. The mice treated with a single *i.p.* dose of C75 (30 mg/kg) showed a body weight loss of 20.1% compared to the control group, as expected (Figure 2). Remarkably, under the same conditions, the EGCG-treated mice (30 mg/kg) did not show any significant weight loss (0.7% of control group). For further confirmation, mice treated with a single *i.p.* dose of EGCG (150 mg/kg, 5-fold the C75 dose) did not show any significant weight loss and appeared healthy after treatment (data not shown). This was consistent with the *in vitro* results of CPT-1 activity shown in Table I.

Discussion

FASN has emerged as a promising drug target for anticancer drug development (8, 9, 11, 12, 31, 32) although specific inhibitors have not been found. Previous independent *in vitro* studies showed that pharmacological blockade of FASN activity using EGCG inhibited the proliferation of human cancer cells *in vitro* (12, 24-26). Additionally, we reported that EGCG-induced cytotoxicity toward three human metastatic breast cancer cell lines (MDA-MB-231, MCF-7 and SK-Br3) expressing different FASN protein levels was directly dependent on the cell FASN level; EGCG potently decreased FASN activity and its ability to inhibit FASN was correlated with the cytotoxic effects on breast cancer cells; EGCG treatment of breast cancer cells produced similar morphological changes (including less dense growth, loss of

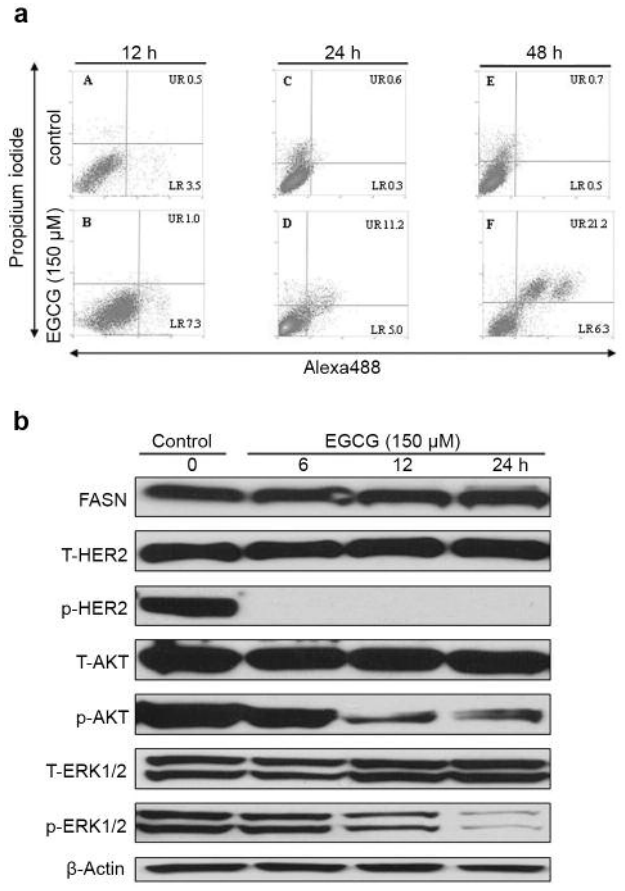

Figure 1. *Effect of EGCG on apoptosis and on HER2-related dowstream signaling pathways in SK-Br3 cells. a) Apoptosis was quantified after 12, 24 or 48 h of EGCG treatment by flow cytometry using an Annexin V-Alexa Fluor 488 (Alexa488) Apoptosis Detection kit. Cells in A, C, and E were control cells; cells in B, D and F were treated with 150 μM EGCG. Cells undergoing early apoptosis are shown in lower right quadrant and late apoptotic cells are shown in the upper right quadrant. Histograms in the figure are from one representative experiment. Equivalent results were obtained in three separate experiments. b) SK-Br3 cells were treated with 150 μM EGCG for the indicated times and were analysed by immunobloting using respective antibodies as described in the Material and Methods section. Blots were reprobed for β-actin as loading control. Gels shown are representative of those obtained from 2 independent experiments.*

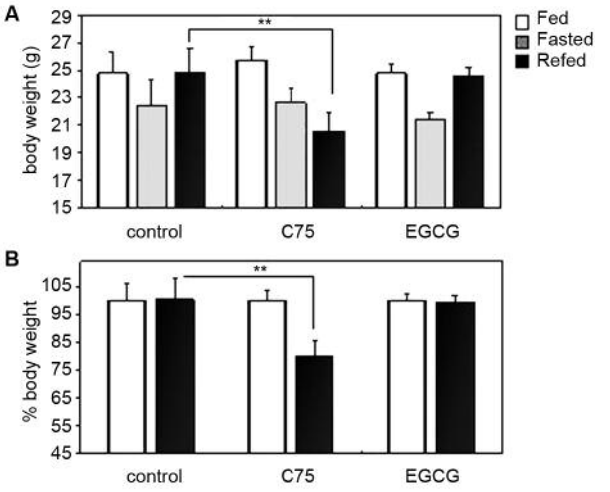

Figure 2. *Effect of EGCG on body weight in vivo. A) Mice were weighed (Fed), fasted for 12 h and weighed (Fasted), treated with a single i.p. 30 mg/kg dose of FASN inhibitor (EGCG or C75) or vehicle (control) as indicated, refed for 24 h and reweighed (Refed). A) Data for body weight are mean±SD (n=5) (**p<0.01 with respect to control). B) Data are expressed as percentage of control body weight and represent mean values±SD for each experimental group (n=5) (**p<0.01 with respect to control).*

cell contact and formation of cellular aggregates) to those observed after siRNA-mediated FASN inhibition (12), implying the reliance of cancer cell survival on FASN expression and activity.

Prior *in vitro* studies from our laboratory with human breast cancer cells using classic FASN inhibitors (cerulenin, C75) showed that HER2/*neu* overexpression and its downstream signaling pathways including phosphatidylinositol 3-kinase (PI3K)/AKT and mitogen-activated protein kinase (MAPK) were linked to FASN-induced cytotoxicity (12, 31, 33) through steroid regulatory element binding protein 1-c (SREBP-1c). FASN blockage using EGCG induced apoptosis and caused a marked decrease in the levels of activated HER2, ERK1/2 and AKT proteins. The present data with EGCG were thus in accordance with those supporting a model in which MAPK and PI3K/AKT activation up-regulates FASN expression in breast, colon, ovarian and prostate cancer cells (34, 35). Future molecular studies of FASN inhibition in cancer cells are likely to explore pathways that modulate apoptosis and continue to search for the biochemical link between FASN inhibition and cancer cell death.

Whereas initial studies with C75, a synthetic FASN inhibitor, showed significant antitumor activity against human xenografts, its therapeutic use was limited by dramatic weight loss (14, 36, 37). C75 is also a stimulator of fatty acid oxidation *via* activation of CPT-1 (12, 15-17), a paradoxical effect considering that FASN inhibition increases levels of malonyl-CoA, the physiological CPT-1 inhibitor (Figure 3). Recently, it has been postulated that C75-induced weight loss ocurred predominantly from its capacity to stimulate CPT-1 and accelerate fatty acid β-oxidation, rather than from a FASN inhibition-dependent mechanism (30). Regarding EGCG, although the effects of extracts of green tea leaves on fatty acid oxidation have been previously investigated (38, 39), this was the first study to analyze the effect of purified EGCG both on fatty acid oxidation regulatory enzyme (CPT-1) *in vitro* and on body weight loss *in vivo*. EGCG did not stimulate CPT-1 activity (≈84%), and in the animal studies, EGCG did not induce any appreciable weight loss after a

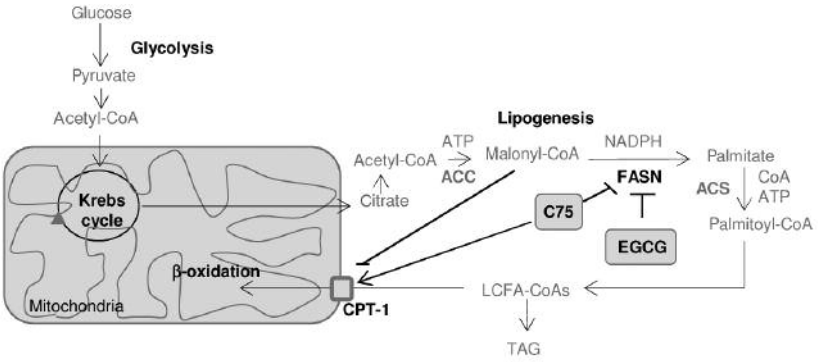

Figure 3. *Fatty acid synthesis and oxidation pathways. Glucose is shunted from the mitochondria to the cytoplasm as citrate, which is converted to acetyl-CoA. Acetyl-CoA is metabolized to malonyl-CoA, which, together with acetyl-CoA and NADPH, are fatty acid synthase (FASN) substrates, which catalyzes the formation of palmitate. Malonyl-CoA inhibitis carnitine palmitoyltransferase-1 (CPT-1), preventing the β-oxidation of the synthesized fatty acids. C75 both blocks FASN activity and stimulates CPT-1 activity. EGCG only blocks FASN activity. ACC, acetyl-CoA carboxylase; ACS, acyl-CoA synthetase; LCFA, long-chain fatty acids; TAG, triacylglycerides.*

single *i.p.* 30 mg/kg or 150 mg/kg dose, compared with the profound weight loss induced by C75 treatment (20.1% of control mice). The present *in vivo* results with C75 were similar to those reported by Thupari *et al.* (15) in which 16% loss of body mass was observed in mice treated with C75 at 20 mg/kg *i.p.* within the first two days of C75 treatment.

In summary, the present results confirmed that EGCG is a potent and specific FASN inhibitor with *in vitro* anticancer activity. Interestingly, the present findings are the first to indicate that EGCG does not exhibit cross-activation of fatty acid oxidation and, most importantly, does not induce animal weight loss, suggesting its potential use as a novel target-directed anticancer drug for future *in vivo* studies, administered alone or in a combination regimen.

Acknowledgements

Financial support was provided by grants from the Susan G. Komen Breast Cancer Foundation (PDF-0504073; R. Colomer), the Spanish Ministerio de Educación y Ciencia, MEC (JCI-2005-001616001-Programa Juan de la Cierva; T. Puig and BFU2007-67322/BMC; P. F. Marrero), the Spanish Society of Medical Oncology (SEOM; R. Colomer and T. Puig) and the Instituto de Salud Carlos III (FIS PI04/1417; R. Colomer, RD06-0020-0028; R. Colomer and ISCIII-RETIC RD06; D. Haro).

References

1 Maier T, Jenni S and Ban N: Architecture of mammalian fatty acid synthase at 4.5 A resolution. Science *311*: 1258-1262, 2006.
2 Epstein JI, Carmichael M and Partin AW: OA-519 (fatty acid synthase) as an independent predictor of pathologic state in adenocarcinoma of the prostate. Urology *45*: 81-86, 1995.
3 Milgraum LZ, Witters LA, Pasternak GR and Kuhajda FP: Enzymes of the fatty acid synthesis pathway are highly expressed in *in situ* breast carcinoma. Clin Cancer Res *3*: 2115-2120, 1997.
4 Kuhajda FP: Fatty-acid synthase and human cancer: new perspectives on its role in tumor biology. Nutrition *16*: 202-228, 2000.
5 Swinnen JV, Roskams T, Joniau S, Van Poppel H, Oven R, Baert L *et al*: Overexpression of fatty acid synthase is an early and common event in the development of prostate cancer. Int J Cancer *98*: 19-22, 2002.
6 Takahiro T, Shinichi K and Toshimitsu S: Expression of fatty acid synthase as a prognostic indicator in soft tissue sarcomas. Clin Cancer Res *9*: 2204-2212, 2003.
7 Visca P, Sebastiani V, Botti C, Diodoro MG, Lasagni RP, Romagnoli F *et al*: Fatty acid synthase (FAS) is a marker of increased risk of recurrence in lung carcinoma. Anticancer Res *24*: 4169-4173, 2004.
8 Kuhajda FP, Pizer ES, Li JN, Mani NS, Frehywot GL and Townsend CA: Synthesis and antitumor activity of an inhibitor of fatty acid synthase. Proc Natl Acad Sci USA *97*: 3450-3454, 2000.
9 Pizer ES, Jackisch C, Wood FD, Pasternak GR, Davidson NE and Kuhajda FP: Inhibition of fatty acid synthesis induces programmed cell death in human breast cancer cells. Cancer Res *56*: 2745-2747, 1996.
10 Menendez JA, Colomer R and Lupu R: Inhibition of tumor-associated fatty acid synthase activity enhances vinorelbine (Navelbine)-induced cytotoxicity and apoptotic cell death in human breast cancer cells. Oncol Rep *12*: 411-422, 2004.
11 Zhao W, Kridel S, Thorburn A, Kooshki M, Little J, Hebbar S *et al*: Fatty acid synthase: a novel target for antiglioma therapy. Br J Cancer *95*: 869-878, 2006.
12 Puig T, Vazquez-Martin A, Relat J, Petriz J, Menendez JA, Porta R *et al*: Fatty acid metabolism in breast cancer cells: differential inhibitory effects of epigallocatechin gallate (EGCG) and C75. Breast Cancer Res Treat *109*: 471-479, 2007.
13 Alli PM, Pinn ML, Jaffee EM, Mac Fadden JM and Kuhajda FP: Fatty acid synthase inhibitors are chemopreventive for mammary cancer in *neu*-N transgenic mice. Oncogene *24*: 39-46, 2005.
14 Loftus TM, Jaworsky DE, Frehywot GL, Townsted CA, Ronnet GV, Lane MD *et al*: Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. Science *288*: 2379-2381, 2000.

15 Thupari JN, Landry LE, Ronnet GV and Kuhajda FP: C75 increases peripheral energy utilization and fatty acid oxidation in diet-induced obesity. Proc Natl Acad Sci USA *99*: 9498-9502, 2002.
16 Nicot C, Napal L, Relat J, Marrero PF and Haro D. C75 activates malonyl-CoA sensitive and insensitive components of the CPT system. Biochem Biophys Res Commun *325*: 660-664, 2004.
17 Yang N, Jais JS, Skillman TL, Burris L, Seng TW and Hammond C: C75 [4-methylene-2-octyl-5-oxo-tetrahydro-furan-3-carboxylic acid] activates carnitine palmitoyltransferase-1 in isolated mitochondria and intact cells without displacement of bound malonyl CoA. J Pharmacol Exp Ther *312*: 127-133, 2005.
18 McGarry JD and Brown NF: The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur J Biochem *244*: 1-14, 1997.
19 Aja S, Landree LE, Kleman AM, Medghalchi SM, Vadlamudi A, McFadden JM *et al*: Pharmacological stimulation of brain carnitine palmitoyl-transferase-1 decreases food intake and body weight. Am J Physiol Regul Integr Comp Physiol *294*: R352-361, 2004.
20 Fujiki H, Sugamuna M, Imai K and Nakachi K: Green tea: cancer preventive beverage and/or drug. Cancer Lett *188*: 9-13, 2002.
21 Adebamowo CA, Cho E, Sampson L, Katan MB, Spiegelman D, Willet WC *et al*: Dietary flavonols and flavonol-rich foods intake and the risk of breast cancer. Int J Cancer *114*: 628-33, 2005.
22 Vergote D, Cren-Olive C, Chopin, Toillon RA, Rolando C, Hondermarck H *et al*: (–)-Epigallocatechin (EGC) of green tea induces apoptosis of human breast cancer cells but not of their normal counterparts. Breast Cancer Res Treat *76*: 195-201, 2002.
23 Jung YD and Ellis LM: Inhibition of tumour invasion and angiogenesis by epigallocatechin gallate (EGCG), a major component of green tea. Int J Exp Pathol *82*: 309-316, 2001.
24 Khan N, Afaq F, Saleem M, Ahmad N and Mukthar H: Targeting multiple signaling pathways by green tea polyphenol (–)-epigallocatechin-3-gallate. Cancer Res *66*: 2500-2505, 2006.
25 Yeh CW, Chen WJ, Chiang CT, Lin-Shiau SY and Lin JK: Suppression of fatty acid synthase in MCF-7 breast cancer cells by tea and tea polyphenols: a possible mechanism for their hypolipidemic effects. Pharmacogenomics *3*: 267-276, 2003.
26 Brusselmans K, Heyns W, Verhoeven G and Swinnen JV: Epigallocatechin-3-gallate is a potent natural inhibitor of fatty acid synthase in intact cells and selectively induces apoptosis in prostate cancer cells. Int J Cancer *106*: 856-8562, 2003.
27 Brusselmans K, De Schrijver E, Verhoeven G and Swinnen JV: RNA interference-mediated silencing of the acetyl-CoA-carboxylase-alpha gene induces growth inhibition and apoptosis of prostate cancer cells. Cancer Res *65*: 6719-6725, 2005.
28 Shimizu M, Deguchi A, Lin JT, Moriwaki H, Kopelovich L and Weinstein IB: (–)-Epigallocatechin gallate and polyphenon E inhibit growth and activation of the epidermal growth factor receptor and human epidermal growth factor receptor-2 signaling pathways in human colon cancer cells. Clin Cancer Res *11*: 2735-2746, 2005.
29 Baliga MS, Meleth S and Katiyar SK: Growth inhibitory and antimetastatic effect of green tea polyphenols on metastasis-specific mouse mammary carcinoma 4T1 cells in *in vitro* and *in vivo* systems. Clin Cancer Res *11*: 1918-1927, 2005.
30 Gao S, Lane MD. Effect of the anorectic fatty acid synthase inhibitor C75 on neuronal activity in the hypothalamus and brainstem. Proc Natl Acad Sci USA *100*: 5628-5633, 2003.
31 Menendez JA, Lupu R and Colomer R: Targeting fatty acid synthase: potential for therapeutic intervention in *her-2/neu*-overexpressing breast cancer. Drug News Perspect *18*: 375-385, 2005.
32 Kuhajda FP: Fatty acid synthase and cancer: new application of an old pathway. Cancer Res *66*: 5977-5980, 2006.
33 Menendez JA, Vellon L, Mehmi I, Oza BP, Colomer R and Lupu R: Inhibition of fatty acid synthase (FAS) suppresses *HER2/neu* (erbB-2) oncogene overexpresion in cancer cells. Proc Natl Acad Sci USA *101*: 10715-10720, 2005.
34 Van de Sande T, De Schrijver E, Heyns W, Verhoeven G and Swinnen JV: Role of the phosphatidylinositol 3'-kinase/PTEN/Akt kinase pathway in the overexpression of fatty acid synthase in LNCaP prostate cancer cells. Cancer Res *62*: 642-646, 2002.
35 Wang HQ, Altomare DA, Skele KL, Paulikakos PI, Kuhajda FP, Di Cristofano A *et al*: Positive feedback regulation between AKT activation and fatty acid synthase expression in ovarian carcinoma cells. Oncogene *24*: 3574-3582, 2005.
36 Tu Y, Thupari JN, Kim EK, Pinn ML, Moran TH, Ronnet GV *et al*: C75 alters central and peripheral gene expression to reduce food intake and increase energy expenditure. Endocrinology *146*: 486-493, 2005.
37 Kim EK, Miller I, Aja S, Landree LE, Pinn L, Mac Fadden J *et al*: C75, a fatty acid synthase inhibitor, reduces food intake *via* hypothalamic AMP-activated protein kinase. J Biol Chem *279*: 19970-19976, 2004.
38 Ikeda I, Hamamoto R, Uzu K, Imaizumi K, Nagao K, Yanagita T *et al*: Dietary gallate esters of tea catechins reduce deposition of visceral fat, hepatic triacylglycerol, and activities of hepatic enzymes related to fatty acid synthesis in rats. Biosci Biotechnol Biochem *69*: 1049-1053, 2005.
39 Murase T, Haramizu S, Shimotoyodome A, Nagasawa A and Tokimitsu I: Green tea extract improves endurance capacity and increases muscle lipid oxidation in mice. Am J Physiol Regul Integr Comp Physiol *288*: 708-715, 2005.

Received May 16, 2008
Revised July 16, 2008
Accepted August 14, 2008