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ORIGINAL ARTICLE

Apoptotic effect of tannic acid on fatty acid synthase over-expressed human breast cancer cells

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Abstract Breast cancer is one of the most common cancers and is the second leading cause of cancer mortality in women worldwide. Novel therapies and chemo-therapeutic drugs are urgently needed to be developed for the treatment of breast cancer. Increasing evidence suggests that fatty acid synthase (FAS) plays an important role in breast cancer, for the expression of FAS is significantly higher in human breast cancer cells than in normal cells. Tannic acid (TA), a natural polyphenol, possesses significant biological functions, including bacteriostasis, hemostasis, and anti-oxidant. Our previous studies demonstrated that TA is a natural FAS inhibitor whose inhibitory activity is stronger than that of classical FAS inhibitors, such as C75 and cerulenin. This study further assessed the effect and therapeutic potential of TA on FAS over-expressed breast cancer cells, and as a result, TA had been proven to possess the functions of inhibiting intracellular FAS activity, down-regulating FAS expression in human breast cancer MDA-MB-231 and MCF-7 cells, and inducing cancer cell apoptosis. Since high-expressed FAS is recognized as a molecular marker for breast cancer and plays an important role in cancer prognosis, these findings suggest that TA is a potential drug candidate for treatment of breast cancer.

Keywords Tannic acid · Fatty acid synthase · Inhibit · Apoptosis · Breast cancer cell

Introduction

Worldwide, breast cancer accounts for 22.9 % of all cancers (excluding non-melanoma skin cancers) in women [1]. In early 2012, there were nearly three million breast cancer patients in the United States, with more than two hundred thousand additional women estimated to develop breast cancer in that same year [2]. Precaution and improved adjuvant treatments have increased breast cancer survival rates since the 1970s, with current 5-year survival rates at nearly 90 % [2]. Despite these significant improvements, the chances of recurrent or relapsed disease are sobering. In women at increased risk of breast cancer, drugs such as tamoxifen and raloxifene have been shown to reduce the risk, but with their own toxicity and side effects. Consequently, increasing attention has been paid on the naturally occurring chemopreventive agents, particularly those present in dietary and medicinal plants due to their bioactive substances and relative low toxicity [3].

Tannic acid (TA) exists widely in fruits, cereals, legumes, herbs, and vegetable drinks, including tea, red wine, coffee, etc. With a chemical formula of $C_{76}H_{52}O_{46}$, TA is a mixture of polygalloyl glucoses and can be hydrolyzed to glucose and gallic acid. The application of TA on human is restricted because it can form insoluble complexes with precipitate proteins, alkaloids, glycosides, and heavy metals. However, researchers have proved that TA has many biological functions which are applied to improve human health, including treatments of ulcers, burns, hemorrhoids, stomatitis, tonsillitis, and

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pharyngitis. In recent years, extensive researches had focused on the anti-cancer potential of TA. Experiments have demonstrated that TA and related polyphenols inhibited the mouse mammary tumor virus promoter [4, 5]. In addition, TA also inhibits the proliferation of various cancer cell lines [6–8] and induced cancer cell apoptosis [9–12].

Fatty acid synthase (FAS, EC 2.3.1.85), a metabolic enzyme that catalyzes the synthesis of long-chain fatty acids from acetyl-CoA, malonyl-CoA, and NADPH, is expressed at high levels in a variety of human cancer cells, including breast [13, 14], prostate [15, 16], endometrium [17], ovary [18], colon [19], lung [20, 21], and pancreas cancer cells [22]. However, it does not express or expressed at a very low level in normal cells. This expression differential between cancer and normal cells makes FAS a potential diagnostic tumor marker [23], and FAS as a therapeutic target for treating cancer has been identified by various studies with FAS inhibitors [24].

In our previous study, TA was found inhibitory activity on pure FAS in vitro, with a half inhibitory concentration (IC_{50}) value of 0.13 μM [25], which was generally more potent compared with the classical known FAS inhibitors, C75 and EGCG [26]. In this study, we provide evidence for that TA inhibits intracellular FAS activity, down-regulates FAS expression, reduces the amount of intracellular fatty acids and induces apoptosis of breast cancer cells, which contribute a series of mechanism information to its known anti-cancer activities.

Methods

Reagents

Acetyl-CoA, malonyl-CoA, NADPH, DMSO, Hoechst 33258, and TA were purchased from Sigma-Aldrich (St. Louis, MO, USA). FAS antibody for immunoblotting was obtained from BD Biosciences Pharmingen (Shanghai, China). GAPDH antibody was purchased from Cell signaling technology, Inc (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco BRL (Beijing, China). 3-4,5-dimethyl-thiazol-2-yl-2,3-diphenyl tetrazolium bromide (MTT), PBS, and the Trizol reagent were purchased from Invitrogen (Beijing, China). Annexin V-FITC/PI Apoptosis Detection Kit was purchased from Mbchem (Shanghai, China). Hematoxylin and eosin (HE) was purchased from Beyotime (Beijing, China).

Cell lines and culture

Human breast cancer MDA-MB-231 and MCF-7 cell lines were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA). Cells were cultured in DMEM containing 10 % FBS at 37 °C in a humidified atmosphere containing 5 % CO_2.

HE staining

MDA-MB-231 and MCF-7 cells were seeded in 6-well culture dishes (2.5×10^6 cells/well). After treated with TA (0, 2, 4, 8 μM), cells were fixed with paraformaldehyde, washed with distilled water for 2 min and stained with Hematoxylin for 10 min and Eosin for 1.5 min after washing excess dye, and followed by extensive washes with 70 % ethanol. Extracellular matrix was examined under the microscope, and images were captured through ImagePro Plus software (Media Cybernetics, Silver spring, MD).

MTT assay

MDA-MB-231 and MCF-7 cells were cultured in the 96-well plates until over 90 % of them were confluenced, and then were incubated with increasing concentrations of TA for 24 h (37 °C, 5 % CO_2), when the medium was changed to a fresh one within 0.5 mg/ml MTT. After 2-h incubation at 37 °C, the plates were again decanted, and 200 μl DMSO was added to solubilize the formazan crystals present in viable cells. The plate was analyzed at the wavelength of 492 nm by a microplate spectrophotometer (Multiskan, MK3). And wells containing no cells were served as background for the assay. Data were obtained from the average of five experiment wells, and each assay was repeated three times.

Hoechst 33258 staining

MDA-MB-231 and MCF-7 cells were seeded in 6-well culture dishes (2.5×10^6 cells/well). After treatment with TA (0, 2, 4, 8 μM), cells were washed twice with cold PBS, and stained with Hoechst 33258 (5 μg/ml) for 5 min in the dark, then followed by extensive washes. Nuclear staining was examined under the fluorescence microscope and images were captured using ImagePro Plus software.

Western blotting

MDA-MB-231 and MCF-7 cells were cultured in D-10-cm dishes until 90 % confluence and then treated with TA (0, 1, 2, 4 μM) for 24 h. Cells were washed with cold PBS and lysed by incubation in lysis buffer, incubated on ice for 1 min, collected with cell scraper, then were centrifuged at 12,000 rpm for 10 min at 4 °C. Total protein concentrations were determined by using bicinchoninic acid (BCA) assay (Pierce). Proteins were separated by SDS-PAGE and then transferred

to PVDF membrane and finally analyzed by Western blot according to standard procedures.

Intracellular FAS activity assay

After treating with TA for 24 h, cells were harvested by treatment with trypsin-EDTA solution, pelleted by centrifugation, washed twice, and resuspended in cold PBS. Cells were sonicated at 4 °C and centrifuged at 13, 000 rpm for 30 min at 4 °C to obtain particle-free supernatants. F. 50 μl particle-free supernatant, 25 mM KH_2PO_4-K_2HPO_4 buffer, 0.25 mM EDTA, 0.25 mM dithiothreitol, 30 μM acetyl-CoA, 350 μM NADPH (pH 7.0) in a total volume of 500 μl were monitored at 340 nm for 100 s to measure background NADPH oxidation. After the addition of 100 μM malonyl-CoA, the reaction was assayed for an additional 1 min to determine FAS dependent oxidation of NADPH. FAS activity was expressed in nmoles NADPH oxidized min^{-1} mg $protein^{-1}$.

Detection of cell apoptotic rates by flow cytometry

Apoptosis was determined by staining cells with annexin V-FITC and PI labeling. Briefly, 1.5×10^5 cells/ml were incubated with or without TA (0, 2, 4, 8 μM) for 24 h. Afterwards, the cells were washed twice with ice-cold PBS, and then annexin V-FITC and PI were then applied to stain cells as the kit directions. The status of cell staining was analyzed by using flow cytometer (Becton Dickinson). Viable cells were negative for both PI and annexin V-FITC; apoptotic cells were positive for annexin V-FITC and negative for PI, whereas late apoptotic dead cells displayed strong annexin V-FITC and PI labeling. Non-viable cells, which underwent necrosis, were positive for PI but negative for annexin V-FITC.

Results

### TA caused MDA-MB-231 and MCF-7 cells morphological changes

The morphological changes of MDA-MB-231 and MCF-7 cells were examined by HE staining. As shown in Fig. 1, cells adhered well and displayed normal morphology in the control cells, whereas shrinkage and loose were observed in TA treated cells. Moreover, the shrinkage became progressively larger with increasing concentrations of TA. After treated with 4 and 8 μM TA, the majority of cells became shrunken, and were observed to float in the culture medium, demonstrating that TA inhibited cancer cell growth in a dose-dependent manner. Since TA is an inhibitor of FAS, the important enzyme to catalyze the synthesis of fatty acids which are essential for cell membranes establishment, the cell morphological changes caused by TA may be related to the permeability of cell membranes, membrane protein distribution, and apoptosis or necrosis. Based on these speculations, we conducted the next experiments.

TA showed time- and dose-dependent reduction on the viability of MDA-MB-231 and MCF-7 cells

Cells were incubated with MTT for 2 h at 37 °C, and reaction was stopped by adding 150 μl DMSO. The absorbance was then measured after the MTT formazan extracted from the cells and thoroughly solubilized. The MTT assay showed that TA reduced cell viability in a dose- and time-dependent manner (Fig. 2). In MDA-MB-231 cells when treated with TA for 6, 12, 18, and 24 h, the IC_{50} values were 5.8, 5.0, 3.5, and 2.5 μM, respectively. While for MCF-7 cells, when treated with TA for 6, 12, 18, and 24 h, the IC_{50} values were >10, 8.0, 6.0, and 4.0 μM, respectively. These results indicated that TA had a similar effect on both MDA-MB-231 and MCF-7 cells, although the inhibitory strengths on them were different.

Fig. 1 TA induced morphologic changes in MDA-MB-231 and MCF-7 cells. A representative HE staining of MDA-MB-231 and MCF-7 cells after treating with TA (0, 2, 4, 8 μM) for 24 h. After TA treatment, cells became shrinkage and loose. Original magnification, ×100
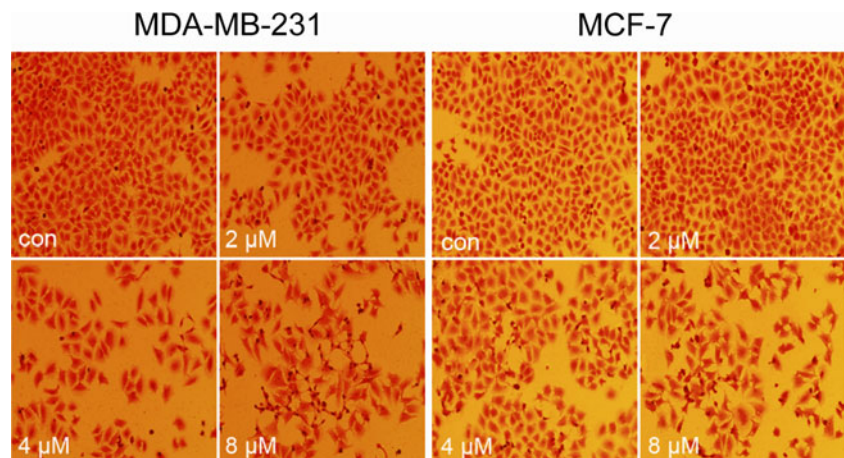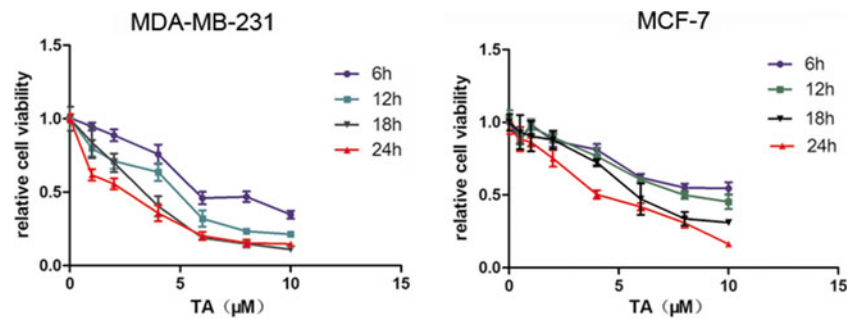
Fig. 2 Inhibitory effect of TA on the viability of MDA-MB-231 and MCF-7 cells. MDA-MB-231 and MCF-7 cells were pretreated with 0.1 % DMSO or various concentrations of TA for 6, 12, 18, and 24 h, and cell viability assays were performed using the MTT method. Results were expressed as relative cell viability as compared with untreated control. The data were expressed as the mean±SD of three independent experiments

TA induced MDA-MB-231 and MCF-7 cells apoptosis

In order to verify whether TA induced cell apoptosis or necrosis, we used Hoechst 33258 staining and flow cytometry with annexin V and PI staining. As shown in Fig. 3a, Hoechst 33258 stained cells exhibited more fluorescence as the concentration of added TA increased which implied that cell membrane permeability was enhanced. According to the concentrations of TA, treated cells were cultured separately, and then cell apoptosis was detected by flow cytometry. As shown in Fig. 3b, when treated with different concentration of TA (2.5, 5, 10 μM), the ratio of early apoptosis was 17.25, 20.60, and 28.00 % and late apoptosis was 18.23, 37.35, and 48.21 % in MDA-MB-231 cells. As for MCF-7 cells, the ratio was 6.70, 21.48, and 22.58 % in early apoptosis and 27.85, 44.36, and 54.05 % in late apoptosis. These results indicated that TA induced MDA-MB-231 and MCF-7 cells apoptosis in a dose-dependent manner.

Fig. 3 TA induced apoptosis in MDA-MB-231 and MCF-7 cells. **a** Cell culture was performed as described in "Methods" section. Photos of MDA-MB-231 and MCF-7 cells were stained with Hoechst 33258 for 30 min in the dark to examine the cleaved nuclei, which is a sign of apoptosis. The concentrations of TA were 0, 2, 4, and 8 μM. Original magnification, ×100. **b** Apoptosis was evaluated using an annexin V-FITC apoptosis detection kit and flow cytometry. The *x*- and *y-axes* represent annexin V-FITC staining and PI, respectively. The representative pictures were from MDA-MB-231 and MCF-7 cells incubated with different concentrations of TA (0, 2.5, 5, 10 μM)
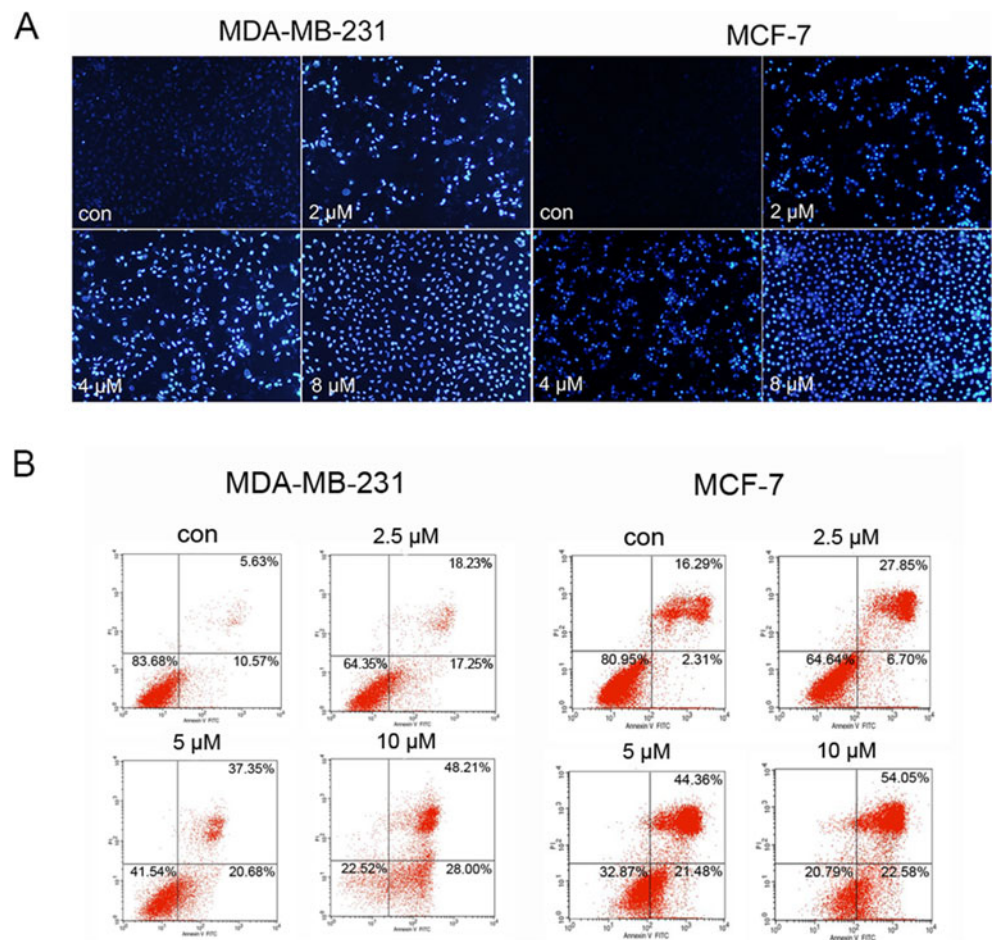
TA suppressed FAS expression and inhibited intracellular FAS activity

We already proved that TA had a potent inhibition to FAS in vitro; however, the effect of TA on intracellular FAS has not been clarified yet. So, we designed the experiment to determine the effect of TA on intracellular FAS. Compared with control, TA not only down-regulated FAS expression (Fig. 4a) but also inhibited intracellular FAS activity with a dose-dependent manner (Fig. 4b). After treating with 1, 2, and 4 μM TA separately for 24 h, the intracellular FAS activities of MDA-MB-231 cells were reduced by 64.04, 48.98, and 36.73 %, correspondingly. And similarly, the intracellular FAS activities of MCF-7 cells were reduced by 91.11, 62.22, and 53.33 %, respectively.

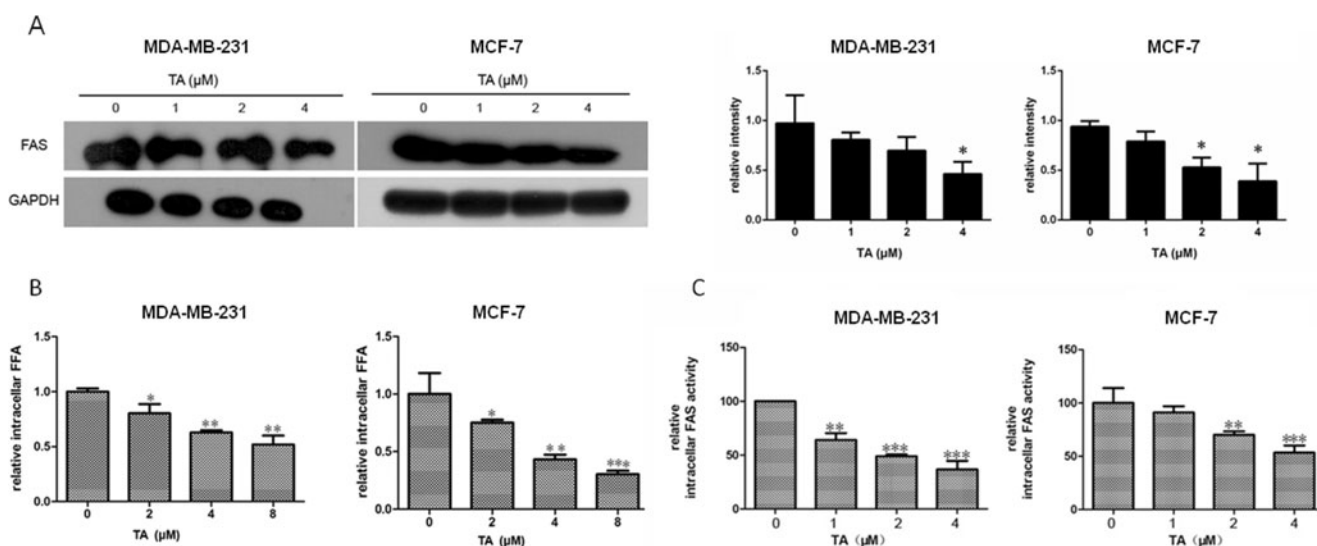## TA reduced the amount of fatty acids in MDA-MB-231 and MCF-7 cells

The amount of intracellular fatty acids in MDA-MB-231 and MCF-7 cells treated with 2, 4, and 8 μM TA were measured, and results (Fig. 4c) showed that the amount of intracellular fatty acids in treated cells decreased dose-dependently. As intracellular fatty acids were synthesized by FAS, these results further indicated that TA indeed had an impact on intracellular FAS. Above results demonstrated that TA induced cell apoptosis via targeting FAS.

Discussion

Breast cancer is a main malignancy and a leading cause of cancer deaths among women worldwide [1, 2], though there are some therapies including surgery, chemotherapy, radiology, and biological therapy. As effective drugs with low side effect are badly needed, herbs and dietary supplements are being studied to find out if they might help to reduce the risk. However, few has been shown to have satisfied activity.

FAS is a key enzyme participating in de novo lipogenesis and plays an important role in converting excess carbon intake into fatty acids for energy storage [14, 27]. Compared with normal tissue, FAS expression levels are significantly high in rapidly proliferating cells, such as breast, liver, prostate, ovarian, endometrial, thyroid cancer cells [24, 28], which suggests that tumors need more fatty acids for growth than can be acquired from the circulation. The anti-cancer potential of FAS inhibitors was recognized as early as 2000 when C75, a well-known FAS inhibitor, has been shown to possess potent anti-cancer activity both in vitro and in vivo. It is necessary to search for more FAS inhibitors that may be applied practically in treatment of cancer.

TA is a common tannin existed in tea, coffee, immature fruits, etc. and has also been used as a food additive. Through our previous screening, TA was found to be a potent FAS inhibitor. Based on the evidence that FAS is over-expressed in cancer cells and TA is a novel FAS inhibitor, we hypothesize that TA is a potential targeted drug for cancer treatment.

Fig. 4 Effects of TA on FAS expression, intracellular FAS activity, and the amount of fatty acids in MDA-MB-231 and MCF-7 cells. **a** Representative pictures and density analysis of protein bands for FAS protein expression by Western blot analysis. Cells were treated with 0, 1, 2, and 4 μM TA. GAPDH was used as a control. TA down-regulated FAS expression in a dose-dependent manner. **b** FAS activity assay was described in "Methods" section. Cells were treated with 0, 1, 2, and 4 μM TA. Data were normalized to control cells without TA (0 μM). Relative

FAS activities were represented as the means±SD from three independent experiments with similar results. **p<0.01 and ***p<0.001 compared to the control (0 μM). **c** MDA-MB-231 and MCF-7 cells were treated with TA at various concentrations (0, 2, 4, 8 μM) for 24 h. And then, the amount of intracellular fatty acid was determined by Fatty Acid Assay Kit. Data were expressed as means±SD (n=3). *p<0.05, **p<0.01, and ***p<0.001 significantly different from control (0 μM)

Researchers have previously reported that FAS was over-expressed in MDA-MB-231 and MCF-7 cells [29, 30], so these two breast cancer cell lines were selected to testify the inhibitory capability of TA. Consistent with our expectations, TA did inhibit intracellular FAS expression and activity in breast cancer cells. However, we found that TA had a stronger effect on intracellular FAS activity than on FAS expression, the mechanism of which was worth further study. Whether TA could pass smoothly through the cell membrane and exert an effect onto intracellular FAS is also what we need to identify.

FAS is a multifunctional enzyme that catalyzes the synthesis of long-chain saturated fatty acids from acetyl-CoA and malonyl-CoA, in the presence of NADPH, so we measured the amount of intracellular fatty acids. As a result, intracellular fatty acids in TA treated cells dose-dependently decreased, which implied that TA indeed affected the lipid metabolism. Moreover, result of Hoechst staining showed that the permeability of cell membranes increased. Based on these phenomena, we hypothesized that the lipid metabolism disorder, which related to the permeability of cell membranes and energy metabolism in cells, may lead to cell apoptosis.

Booth and colleagues have previously reported that TA-crosslinked collagen Type I beads induced apoptosis in MDA-MB-231 and MCF-7 cells [31]. They found that MCF-7 cells were more sensitive to the effects of TA, as lower concentrations of TA were able to induce elevated levels of the activated caspases 9 and 3/7 compared to the triple negative MDA-MB-231 breast cancer cells, which suggested that ER positive breast cancer cells were more susceptible to the effects of TA. However, in the present work, we found that TA showed apoptotic effect on both ER positive and ER negative cells.

Although the detailed mechanism of TA induced apoptosis has not been fully clarified, we proposed that a possible way may be that TA inhibits FAS activity and reduces the amount of free fatty acids, which are the main material for the synthesis of membrane phospholipids of cancer cells. Interestingly, compared with our previous results, TA showed stronger toxicity on MDA-MB-231 and MCF-7 cells than on FAS over-expressed 3 T3-L1 adipocytes [25]. It revealed that in an appropriate concentration, TA may induce cancer cells apoptosis and have little cytotoxic to adipocytes. This result provided the possibility for the utility of TA to be explored as an active and low toxic drug candidate for preventing and treating cancer.

Moreover, we have already proved that the FAS activity inhibited by TA would not induce non-specific FAS sedimentation [25]. In the present research, the highest concentration of TA used was much lower than that could induce FAS sedimentation, which guaranteed that the inhibition of TA on cancer cells have no relation to FAS non-specific sedimentation. While in chemotherapy, toxicity and drug-resistance are main obstacles in achieving and maintaining a cancer-free status [32–34]. As a result, anti-cancer agents have evolved to molecular-targeting agents that selectively and effectively destroy cancer cells other than indiscriminately killing them.

In conclusion, TA could induce human breast cancer cells apoptosis by inhibiting intracellular FAS activity and down-regulating FAS expression. Our results suggest that TA can be applied in treatment of breast cancer, and it may supply useful ideas and new clues in developing target-directed anti-cancer drugs for further studies.

Conflicts of interest None

References

1. Jemal A, Bray F, Center MM. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.

2. Siegel R, DeSantis C, Virgo K, Stein K, Mariotto A, Smith T, Cooper D, Gansler T, Lerro C, Fedewa S, LinC, Leach C, Cannady RS, Cho H, Scoppa S, Hachey M, Kirch R, Jemal A, Ward E Cancer treatment and survivorship statistics, 2012. CA Cancer J Clin. 2012;62:220–41.

3. Sebastian KS, Thampan RV. Differential effects of soybean and Fenugreek extracts on the growth of MCF-7 cells. Chem Biol Interact. 2007;17:135–43.

4. Abe I, Umehara K, Morita R. Green tea polyphenols as potent enhancers of glucocorticoid-induced mouse mammary tumor virus gene expression. Biochem Biophys Res Commun. 2001;281:122–5.

5. Uchiumi F, Sato T, Tanuma S. Identification and characterization of a tannic acid-responsive negative regulatory element in the mouse mammary tumor virus promoter. J Biol Chem. 1998;273:12499–508.

6. Jiang Y, Satoh K, Aratsu C, Kobayashi N, Unten S, Kakuta H, Kikuchi H, Nishikawa H, Ochiai K, SakagamiH Combination effect of lignin F and natural products. Anticancer Res. 2001;21:965–70.

7. Devi MA, Das NP. In vitro effects of natural plant polyphenols on the proliferation of normal and abnormal human lymphocytes and their secretions of interleukin-2. Cancer Lett. 1993;69:191–6.

8. Ramanathan R, Tan CH, Das NP. Cytotoxic effect of plant polyphenols and fat-soluble vitamins on malignant human cultured cells. Cancer Lett. 1992;62:217–24.

9. Sakagami H, Jiang Y, Kusama K, Atsumi T, Ueha T, Toguchi M, Iwakura I, Satoh K, Ito H, Hatano T,Yoshida T Cytotoxic activity of hydrolyzable tannins against human oral tumor cell lines—a possible mechanism. Phytomedicine. 2000;7:739–47.

10. Pan MH, Lin JH, Lin-Shiau SY, Lin JK Induction of apoptosis by penta-O-galloyl-β-D-glucose through activation of caspase-3 in human leukemia HL-60 cells. Eur J Pharmacol. 1999;381:171–83.

11. Wang CC, Chen LG, Yang LL. Cuphiin D1, the macrocyclic hydrolyzable tannin induced apoptosis in HL- 60 cell line. Cancer Lett. 2000;149:77–83.

12. Yang LL, Lee CY, Yen KY. Induction of apoptosis by hydrolyzable tannins from Eugenia jambos L. on human leukemia cells. Cancer Lett. 2000;157:65–75.

13. Alo PL, Visca P, Marci A, Mangoni A, Botti C, Di Tondo U Expression of fatty acid synthase (FAS) as a predictor of recurrence in stage I breast carcinoma patients. Cancer. 1996;77:474–82.

14. Milgraum LZ, Witters LA, Pasternack GR, Kuhajda FP Enzymes of the fatty acid synthesis pathway are highly expressed in in situ breast carcinoma. Clin Cancer Res. 1997;3:2115–20.

15. Epstein JI, Carmichael M, Partin AW. OA-519 (fatty acid synthase) as an independent predictor of pathologic state in adenocarcinoma of the prostate. Urology. 1995;45:81–6.

16. Swinnen JV, Roskams T, Joniau S, Van Poppel H, Oyen R, Baert L, Heyns W, Verhoeven G Overexpression of fatty acid synthase is an early and common event in the development of prostate cancer. Int J Cancer. 2002;98:19–22.

17. Pizer ES, Lax SF, Kuhajda FP, Pasternack GR, Kurman RJ Fatty acid synthase expression in endometrial carcinoma: correlation with cell proliferation and hormone receptors. Cancer. 1998;83:528–37.

18. Gansler TS, Hardman W, Hunt DA, Schaffel S, Hennigar RA Increased expression of fatty acid synthase (OA-519) in ovarian neoplasms predicts shorter survival. Hum Pathol. 1997;28:686–92.

19. Rashid A, Pizer ES, Moga M, Milgraum LZ, Zahurak M, Pasternack GR, Kuhajda FP, Hamilton SR Elevated expression of fatty acid synthase and fatty acid synthetic activity in colorectal neoplasia. Am J Pathol. 1997;150:201–8.

20. Visca P, Sebastiani V, Botti C, Diodoro MG, Lasagni RP, Romagnoli F, Brenna A, De Joannon BC, Donnorso RP, Lombardi G, Alo PL Fatty acid synthase (FAS) is a marker of increased risk of recurrence in lung carcinoma. Anticancer Res. 2004;24:4169–73.

21. Orita H, Coulter J, Tully E, Kuhajda FP, Gabrielson E Inhibiting fatty acid synthase for chemoprevention of chemically induced lung tumors. Clin Cancer Res. 2008;14:2458–64.

22. Alo PL, Amini M, Piro F, Pizzuti L, Sebastiani V, Botti C, Murari R, Zotti G, Di Tondo U Immunohistochemical expression and prognostic significance of fatty acid synthase in pancreatic carcinoma. Anticancer Res. 2007;27:2523–7.

23. Walter K, Hong SM, Nyhan S, Canto M, Fedarko N, Klein A, Griffith M, Omura N, Medghalchi S, KuhajdaF, Goggins M Serum fatty acid synthase as a marker of pancreatic neoplasia. Cancer Epidem Biomar. 2009;18:2380–5.

24. Kuhajda FP. Fatty acid synthase and cancer: new application of an old pathway. Cancer Res. 2006;66:5977–80.

25. Fan HJ, Wu D, Tian WX, Ma XF Inhibitory effects of tannic acid on fatty acid synthase and 3T3-L1 preadipocyte. Biochimica et Biophysica Acta. 1831;2013:1260–6.

26. Wang X, Tian W. Green tea epigallocatechin gallate: a natural inhibitor of fatty-acid synthase. Biochem Biophys Res Commun. 2001;288:1200–6.

27. Aggarwal BB, Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol. 2006;71: 1397–421.

28. Choi WI, Jeon BN, Park H, Yoo JY, Kim YS, Koh DI, Kim MH, Kim YR, Lee CE, Kim KS, Osborne TF, Hur MW Proto oncogene FBI-1 (Pokemon) and SREBP-1 synergistically activate transcription of fatty-acid synthase gene (FASN). J Biol Chem. 2008;283: 29341–54.

29. Wang Y, Tian WX, Ma XF. Inhibitory effects of onion (Allium cepa L.) extract on proliferation of cancer cells and adipocytes via inhibiting fatty acid synthase. Asian Pac J Cancer Prev. 2012;13: 5573–9.

30. Menendez JA, Mehmi I, Atlas E, Colomer R, Lupu R Novel signaling molecules implicated in tumor-associated fatty acid synthase-dependent breast cancer cell proliferation and survival: role of exogenous dietary fatty acids, p53-p21WAF1/CIP1, ERK1/2 MAPK, p27KIP1, BRCA1, and NF-kappaB. Int J Oncol. 2004;24:591–608.

31. Booth BW, Inskeep BD, Shah H, Park JP, Hay EJ, Burg KJ Tannic Acid preferentially targets estrogen receptor-positive breast cancer. Int J Breast Cancer. 2013;2013:369609.

32. Landis-Piwowar KR, Milacic V, Chen D, Chen D, Yang H, Zhao Y, Chan TH, Yan B, Dou QP The proteasome as a potential target for novel anticancer drugs and chemosensitizers. Drug Resist Updat. 2006;9:263–73.

33. Bendell J, Goldberg RM. Targeted agents in the treatment of pancreatic cancer: history and lessons learned. Curr Opin Oncol. 2007;19:390–5.

34. Carver JR, Shapiro CL, Ng AA, Jacobs L, Schwartz C, Virgo KS, Hagerty KL, Somerfield MR, VaughnDJ ASCO Cancer Survivorship Expert Panel, American Society of Clinical Oncology clinical evidence review on the ongoing care of adult cancer survivors: cardiac and pulmonary late effects. J Clin Oncol. 2007;25:3991–4008.