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The enhancing effect of genistein on apoptosis induced by trichostatin A in lung cancer cells with wild type p53 genes is associated with upregulation of histone acetyltransferase☆

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ABSTRACT

Genistein has been shown to enhance the antitumor activity of trichostatin A (TSA) in human lung carcinoma A549 cells. However, whether the combined treatment exerts the same effect in other lung cancer cells is unclear. In the present study we first compared the enhancing effect of genistein on the antitumor effect of TSA in ABC-1, NCI-H460 (H460) and A549 cells. Second, we investigated whether the effects of genistein are associated with increased histone/non-histone protein acetylation. We found that the enhancing effect of genistein on cell-growth-arrest in ABC-1 cells (p53 mutant) was less than in A549 and H460 cells. Genistein enhanced TSA induced apoptosis in A549 and H460 cells rather than in ABC-1 cells. After silencing p53 expression in A549 and H460 cells, the enhancing effect of genistein was diminished. In addition, genistein increased TSA-induced histone H3/H4 acetylation in A549 and H460 cells. Genistein also increased p53 acetylation in H460 cells. The inhibitor of acetyltransferase, anacardic acid, diminished the enhancing effect of genistein on all TSA-induced histone/p53 acetylation and apoptosis. Genistein in combination with TSA increased the expression of p300 protein, an acetyltransferase, in A549 and NCI-H460 cells. Furthermore, we demonstrated that genistein also enhanced the antitumor effect of genistein in A549-tumor-bearing mice. Taken together, these results suggest that the enhancing effects of genistein on TSA-induced apoptosis in lung cancer cells were p53-dependent and were associated with histone/non-histone protein acetylation.

1. Introduction

Epigenetic aberrations have been strongly implicated in the development of cancers including lung cancer (Petta et al., 2013; Liu et al., 2013). Histone acetyltransferase (HAT) and Histone deacetylases (HDAC) control the acetylation of lysine residues in histone, which lead to chromatin condensation and transcriptional repression. It has been reported that HDAC overexpression leads to silencing of tumor suppressor genes and is associated with carcinogenesis (Petta et al., 2013). In addition, HAT and HDAC may influence the fate of cells through the acetylation of non-histone proteins (Yang and Seto, 2007). Studies have shown that several HDAC inhibitors including trichostatin A (TSA) possess antitumor effects in human lung cancers (Kim et al., 2006; Petta et al., 2013). However, one study found that some NSCLC are sensitive to HDAC inhibitors, such as TSA and vorinostat, while some are not (Miyanaga et al., 2008), which may be due to a difference in genetic makeup.

Genistein is an isoflavone present in soybeans and soybean products. Growing evidence shows dietary isoflavones including genistein may protect against several cancers including lung cancer (Nagata et al., 2007; Li and Chen, 2011). Genistein influences the growth of cancer cells by various possible mechanisms (Caëtano et al., 2006), one of which may be epigenetic regulation (Sarkar et al., 2010; Seo et al., 2011; Dagdemir et al., 2013). It has been reported that genistein significantly increases histone acetylation in vitro (Hong et al., 2004). Our previous studies found that genistein at physiological doses (2–10 μM) enhances the anti-cancer effect of TSA in A549 cells in a dose dependent manner, especially 10 μM genistein synergistically enhances the growth-arrest and apoptosis induced by TSA (Wu et al., 2012). However, whether genistein at these doses exerts the same effect in other human lung cancer cell lines is unclear. In addition, whether the regulation of histone/non-histone protein acetylation contributes to the synergistic antitumor effect of genistein and TSA is also unclear.

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Table 1
The effect of TSA combined with genistein on the growth of human lung cancer cells.

Treatment	Cells					
	Cell number (%)					
	A549		H460		ABC-1	
	24 h	48 h	24 h	48 h	24 h	48 h
C	100 ± 0	133.5 ± 13.4	100 ± 0	157.7 ± 11.7	100 ± 0	116.3 ± 1.2
10 G	98.6 ± 2.1	139.1 ± 11.1	108.7 ± 14.2	125.1 ± 11.0^{ab}	103.9 ± 8.7	95.8 ± 2.6^{a}
TSA 50	84.5 ± 7.5^{a}	73.1 ± 16.1^{b}	88.8 ± 11.1^{a}	79.8 ± 0.6^{a}	98.3 ± 1.65	95.3 ± 8.3^{a}
TSA50 + 5 G	–	–	74.5 ± 12.5^{a}	65.5 ± 0.7	–	–
TSA 50 + 10 G	50.8 ± 16.3^{ab*}	47.5 ± 18.1^{ab*}	56.8 ± 15.3^{ab*}	55.8 ± 7.6^{ab}	95 ± 7.3	85.4 ± 8.7^{a}
TSA 100	–	–	–	–	98.2 ± 7.6	78.8 ± 4.8^{a}
TSA 200	–	–	–	–	81.1 ± 8.9^{a}	71.0 ± 6.9^{a}
TSA 200 + 5 G	–	–	–	–	77.2 ± 1.9^{a}	67.0 ± 6.5^{a}
TSA 200 + 10 G	–	–	–	–	71.1 ± 6.6^{ab*}	63.2 ± 3.9^{ab}

The A549 and H460 cells were incubated with TSA (50 ng/ml) alone or combined with genistein (5 or 10 μM, 5 G and 10 G, respectively) for 24 h and 48 h. The ABC-1 cells were incubated with TSA (50 or 200 ng/ml) alone or combined with genistein for 24 and 48 h. Values are (means ± SD) (n = 3). a: $p < 0.05$ as compared with the control group in the same column. b: $p < 0.05$ as compared with the TSA group (TSA at the same dose) in the same column. *: a significant interaction between TSA and genistein ($p < 0.05$) determined by Two-way analysis. C: control. G: genistein. –: not detected.

Thus, the first aim of the present study was to compare the combined effect of genistein and TSA on the growth-inhibition of three different NSCLC cell lines, A549, NCI-H460 (H640) and ABC-1. According to the study by Miyanaga et al. (2008), A549 and H460 (with wild type p53) are sensitive to HDAC inhibitors while ABC-1 (p53 mutant) is more resistant. Second, we investigated whether genistein enhances the acetylation of histone/non-histone protein induced by TSA and its role in the enhancing effect of genistein. In addition, we investigated whether genistein enhances the antitumor effect of TSA in vivo.

2. Materials and methods

2.1. Cell culture and cell growth test

A549, H460 and ABC-1 cells were cultured in RPMI 1640 containing 10% (*v*/*v*) cosmic calf® serum (Thermo, Massachusetts, USA), 0.37% (*w*/*v*) $NaHCO_3$, penicillin (100 units/mL), and streptomycin (100 μg/mL) at 37 °C in a humidified incubator under 5% CO_2 and 95% air. An equal number (5×10^4/mL) of cells were incubated for 24 h before the various treatments. After being washed twice with phosphate-buffered saline (PBS; pH 7.4, containing 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, and 8.1 mM Na_2HPO_4), the cells were incubated in fresh culture medium containing TSA alone or in combination with genistein, anacardic acid or both. Anacardic acid is an inhibitor of HAT (Balasubramanyam et al., 2003). The medium was replaced every day. Cell growth was measured by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide colorimetric assay (Mosmann, 1983) or Trypan-blue assay (Phillips, 1973). Stock solutions of ethanol–genistein and ethanol–TSA were freshly prepared before each experiment. The final solvent concentration was <0.2%. Control cells were incubated with solvent alone. After being treated for 24 h, the cell number in the control group was taken as 100%, and the cell growth in each treatment at the indicated times was expressed as a percentage (%) of that.

2.2. Apoptosis assay

An Annexin V-FITC apoptosis detection kit (BD Pharmingen, San Diego, CA, USA) was used to determine the number of apoptotic cells. According to the manufacturer's instructions, the treated cells were harvested after the indicated time, washed twice with ice-cold PBS and resuspended in 100 μL of binding buffer. Then an aqueous mixture of Annexin V-FITC and propidium iodide staining buffer was added and the mixture was incubated in the dark at 37 °C for 15 min. Before flow cytometric analysis, 400 μL of binding buffer was added to each sample. A total of 100,000 events per sample were analyzed. Flow cytometric analysis was performed with a FACS Calibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) with WinMDI 2.8 software.

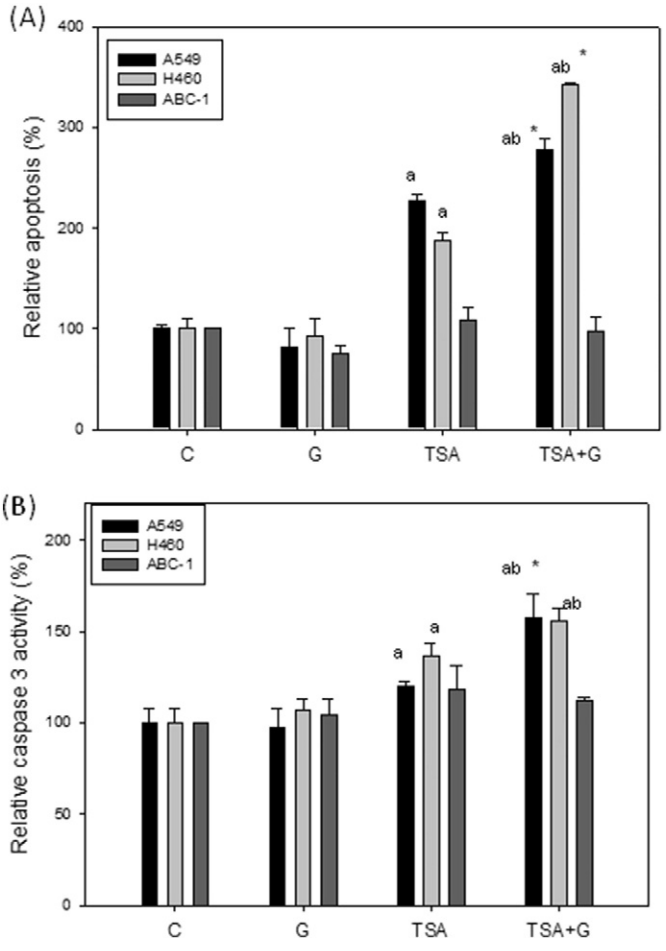

Fig. 1. The effects of TSA alone or in combination with genistein on apoptosis (A) and caspase3 activity (B) in human lung cancer cells. The cells were incubated with TSA alone or in combination with genistein (10 μM) for 48 h. The dose of TSA used in A549 and H460 cells was 50 ng/ml, while 200 ng/ml was used in ABC-1 cells. Values are means ± SD (n = 3). a, b: $p < 0.05$ as compared with the control group and the TSA group, respectively in the same cell line. *: a significant interaction between TSA and genistein ($p < 0.05$) determined by Two-way analysis. C: control. G: genistein.