行政院國家科學委員會專題研究計畫 期末報告

Genistein 促進 trichostatin A 抑制人類非小細胞肺癌細胞生長的體外及體內研究(第 3 年)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 99-2320-B-040-006-MY3
執 行 期 間 ：101 年 08 月 01 日至 102 年 07 月 31 日
執 行 單 位 ：中山醫學大學營養學系（所）

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中 華 民 國 102 年 11 月 07 日

中 文 摘 要 ： 此三年計畫，第一年的研究顯示 genistein (10 贡 M)會增加 Trichostatin A (TSA，一種組蛋白去乙醯酶抑制劑具抗癌效果)所誘發的人類肺腫瘤 A549 細胞組蛋白乙醯化及並透過增加 TNFR1 表現及下游 caspase 蛋白表現增加細胞凋亡。第二年利用不同的肺癌細胞株則發現 genistein 會增加所有肺癌細胞 TSA 誘發組蛋白乙醯化的效果，此效應與 genistein 會增加 actyltransferase 活性有關，但下游基因表現如 p53 蛋白變異之細胞株，genistein 增強 TSA 的抗腫瘤性的效果則較差。第三年的動物研究發現，不同處理方式對於動物體重無顯著影響，只有 TSA+IG(皮下注射 genistein)與控制組相比，能明顯降低異體移植腫瘤裸鼠腫瘤大小。給予 genistein 2 小時後，動物血漿中濃度以 OG(由口給予 genistein)組高於皮下注射組，但在腫瘤組織中 genistein 濃度，則以 IG 高於 OG 處理組。腫瘤組織中 TNFR-1、acetyl histone H3 and H4 表現量以 TSA+IG 組最高。另外，結果發現單獨 TSA 處理能明顯增加 TNF-α 以及 TBARS 含量，IG 能明顯減少 TSA 誘導增加的 TNF-α 以及 TBARS，但 OG 則無此效果。這些體內外研究結果證實 genistein 增加 TSA 抗腫瘤性的效果及部分機制，並增加我們對此一合併處理對抗肺炎效果的了解。

中文關鍵詞： genistein, TSA, 肺癌細胞

英 文 摘 要 ： In the first year, our study demonstrated that through upregulation of TNFR-1death receptor signaling and the activation of caspase protein, genistein enhanced the Trichostatin A (TSA, an anti-tumor drug)-induced apoptosis in A549 cells. In the second year, comparing the enhancing effect of genistein on the antitumor effect of TSA in various lung cancer cell lines, we found that genistien increased TSA-induced histone acetylation in all cell lines. The mechanisms were associated with the activation of acetyltrasferase. However, the down stream protein expression, such as p53 influenced the enhancing effect of genistein on the antitumor effect of TSA. In the third year, using nude mice we performed a mouse xenograft experiment TSA in vivo. The results showed that various treatments did not significantly affect the body weight of nude mice. Only TSA+IG (genistein given intraperitoneally) treatment significantly decreased tumor volume as

compared with the control group. Two hours after genistein administration, the total genistein concentrations in plasma of animals with OG (genistein given orally) treatment were higher than those with IG treatment. However, the total genistein concentrations in tumors of animals with IG treatment were higher than those with OG treatment. Tumors from mice treated with TSA+IG had highest TNFR-1, acetyl histone H3 and H4 levels than other groups. TSA treatment significantly increased the levels of TNF-α in plasma and tumors as well as the level of TBARs in plasma. IG rather than OG significantly decreased the rise of TNF-α and TBARs induced by TSA. Taken together, these in vitro and in vivo studies demonstrated that genistein enhancing the antitumor effect of TSA in lung cancer cells and parts of the mechanisms. These results increase the understanding in the effects of the combined treatment of TSA and genistein in lung cancers.

英文關鍵詞： genistein, TSA, lung cancer cells

一、前言

肺癌是持續多年造成國人惡性腫瘤死亡的主要原因，其治癒率低，五年存活率僅約 10-12%，因此如何有效抑制肺癌已是目前醫療研究重要的方向。Trichostatin A (TSA)，TSA 是一種組蛋白去乙醯化酶抑制劑，藉由抑制組蛋白去乙醯化而使組蛋白維持在高度乙醯化狀態，進而調控基因轉錄，而使癌細胞進行細胞程式性凋亡(Marks et al., 2000. Monneret et al., 2007)。Yuan 等(2010)發現抗癌藥物 TSA 合併營養素-視網酸處理，可有效抑制甲狀腺腫瘤細胞的增生，並降低藥物對細胞的毒性。雖然在過去研究中發現 TSA 可抑制癌細胞生長，但 TSA 之使用、安全劑量及其副作用還需進一步評估。

另外過去的研究發現，天然植物中的植化素(phytochemicals)，例如: 異黃酮- genistein亦具有抑制癌細胞生長的潛力。genistein是一種存於大豆中的植化素，研究顯示genistein可以抑制癌細胞生長，使癌細胞細胞週期停滯於G2/M期，包括：前列腺癌細胞 (Lakshman et al., 2008；Choi et al., 2000)、乳癌細胞(Jeune et al., 2005)、卵巢癌細胞(Gossner et al., 2007)及肺癌細胞(Lian et al., 1998；Gadgeel et al., 2009)等。

本實驗室先前研究發現在肺癌細胞株A549中，TSA合併genistein (5、10 μM)處理可顯著增加肺癌細胞凋亡(Shiau et al., 2010; Wu et al., 2012)，並且細胞中TSA合併genistein (10 μM)時組蛋白乙醯化 (Histone acetylation)現象顯著增加(陳，2007)，但1.其確切的分子機制2.此一現象是否會發生在其他癌細胞中3.在活體中此一合併處理的效果如何目前尚不清楚。

二、目的

基於上述本研究三年的目的分述如下:

1.第一年：

檢測 TNFR1 及 caspase- 2, 3, 8 在 TSA、genistein 單獨或合併處理時其蛋白質與 mRNA 的表現情形，並將轉染(transfection) TNFR1 的 siRNA（small interfering RNA）進入 A549 細胞後，檢測待測試劑對細胞凋亡影響的改變情形，來確定 death receptor pathway 對於 genistein 增強 TSA 誘發 A549 肺癌細胞進行程式性死亡的重要性。

2.第二年：

我們選擇四株對 TSA 敏感性不同的肺癌細胞(A549、H460、ABC-1)，來研究 genistein 對 TSA 抗癌性的增強效果是否在不同的癌細胞均有一致的結果，另外我們也研究 genistein 調節 TSA 誘發的組蛋白乙醯化對其促進 TSA 抗癌性的重要性及可能的機制。

3.第三年：

本年度研究的主要目的是以異種移植模式，研究 genistein 增強 TSA 體內抑制腫瘤細胞生長的效果及機制，並比較 genistein 經口攝入，或與 TSA 同時經靜脈注

射的效果，籍此作為體內濃度效應的評估，以作為日後人體試驗參考的依據。

三、研究方法

1.第一年：

檢測 genistein 是否是透過增強細胞膜上死亡接受器，TNF receptor-1，的表現而增加 TSA 誘發 NSCLC 細胞株，A549 細胞，程式性死亡的效果。A549 細胞以 TSA、genistein 單獨或合併 (6-72 小時)後，分析 A549 細胞生長及 apoptosis 情形，並分別以 RT-PCR 及 western blot assay 分析 TNFR1 表現,以 ELISA kit 分析細胞內 caspase-2、3 及 caspase-8 活性。另外，有些實驗組，種下細胞隔夜後，先給予含 TNF receptor-1 的 siRNA 轉染 24 小時，之後換新鮮培養基並加入 TSA、genistein 單獨或合併培養一段時間後，再進行上述分析。

2.第二年:

依據 Miyanaga 等（2008）的研究及我們先前的預備試驗，我們預備以對 TSA 本身有不同敏感度的細胞株進行試驗，三株細胞分別為：NCI-H460、A549(屬高敏感細胞，我們的預備試驗顯示 TSA 對此二細胞株的 IC50≦1 μM)，及對 TSA-resistant 的細胞 ABC-1 (IC50≧15 μM) (Miyanaga et al., 2008)。我們分別研究 genistein 對 TSA 抗癌性的增強效果，並研究組蛋白乙烯化的表現及 53 蛋白的表現。並加入乙烯轉移 enzyme 抑制劑與細胞共同培養，探討 acetylase 在 genistein 促進效果中的角色。

3. 以異種移種植模式(Hung et al., 2009）進行體內試驗，評估 genistein 體內增加 TSA 抑制 A549 肺腫瘤細胞的效果，並評估 genistein 給予方式的影響，研究分兩部分：

（1） 進行異種移植後的裸鼠，再以靜脈注射給予 TSA、genistein 單獨或二者合併

（2） 進行異種移植後的裸鼠，TSA 從靜脈注射給予，genistein 則由口餵食，藥劑處理方式，同樣的有單獨給予或二者合併。

四、結果討論

1. 第一年(已發表)

REF: Genistein enhances the effect of trichostatin A on inhibition of A549 cell growth by increasing expression of TNF receptor-1. Toxicology and Applied Pharmacology. 2012, 262：247-254 (SCI)

2. 第二年（黃佩茹, 2010）

Table 1、The effect of TSA combined with genistein on the growth of human

lung cancer cells.

	Cells					
	Cell number (%)					
	A549		H460		ABC-1	
Treatment	24H	48H	24H	48H	24H	48H
C	100±0 ^{c}	133.5±13.4 ^{c}	100±0 ^{bc}	157.7±11.7 ^{d}	100±0 ^{c}	116.3±1.2 ^{d}
G	98.6±2.1 ^{c}	139.1±11.1 ^{c}	108.7±14.2 ^{c}	125.1±11.0 ^{c}	103.9±8.7 ^{c}	95.8±2.6 ^{c}
TSA 50	84.5±7.5 ^{b}	73.1±16.1 ^{b}	88.8±11.1 ^{b}	79.8±0.6 ^{b}	98.3±1.65 ^{c}	95.3±8.3 ^{bc}
TSA 50+G	50.8±16.3 ^{a,*}	47.5±18.1 ^{a,*}	56.8±15.3 ^{a,*}	55.8±7.6 ^{a,}	95±7.3 ^{c}	85.4±8.7 ^{b}
TSA 200	NA	NA	NA	NA	81.1±8.9 ^{b}	71.0±6.9 ^{a}
TSA 200+G	NA	NA	NA	NA	71.1±6.6 ^{a,*}	63.2±3.9 ^{a,}

The A549 and H460 cells were incubated with TSA (50 ng/ml) alone or combined with genistein (10 μM) for 24h and 48h. The ABC-1 cells were incubated with TSA (50 or 200 ng/ml) alone or combined with genistein (10 μM) for 24 and 48h. Values (means±SD) in each cell line at the same time not sharing a common letter are significantly different ($p<0.05$). Two-way analysis was performed to determine the interaction between TSA and genistein. * denotes a significant interaction between TSA and genistein ($p<0.05$). C: control. G: genistein. NA: not available.

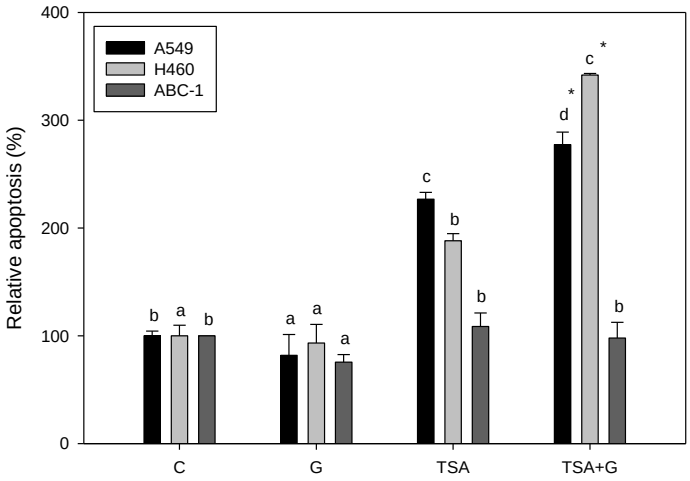


Figure 1、The effects of TSA alone or combined with genistein on apoptosis of human lung cancer cells. The A549 and H460 cells were incubated with TSA (50 ng/ml) alone or combined with genistein (10 μM) for 48h. The ABC-1 cells were incubated with TSA (200 ng/ml) alone or combined with genistein (10 μM) for 48h. Values (means±SD) among the same cell line not sharing a common letter are significantly different ($p<0.05$). Two-way analysis was performed to determine the interaction between TSA and genistein. * denotes a significant interaction between TSA and genistein ($p<0.05$). C: control. G: genistein.

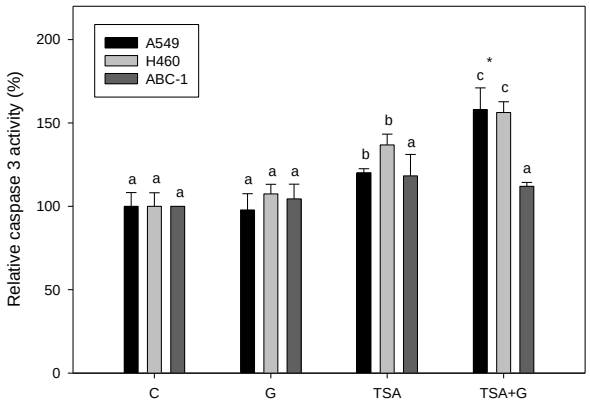
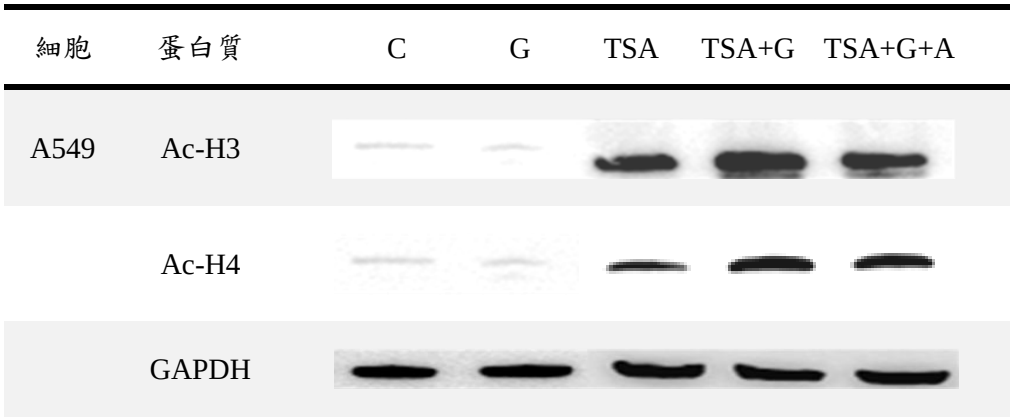
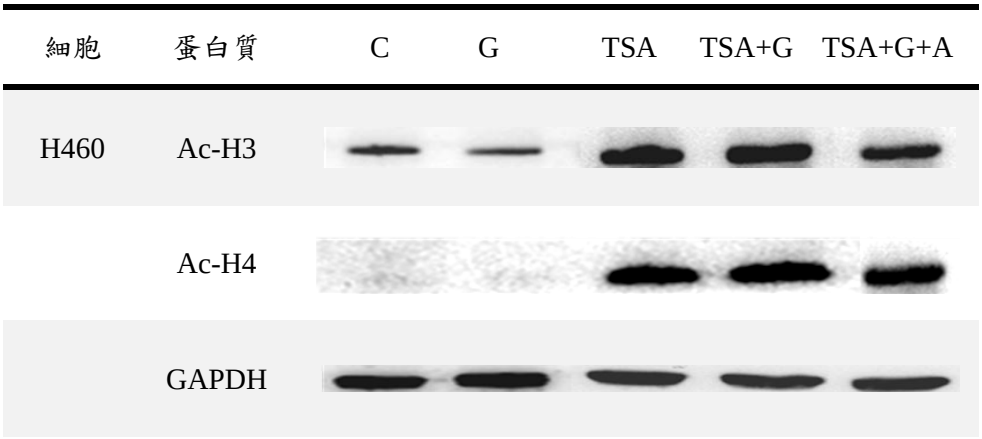


Figure 2、The effects of TSA alone or combined with genistein on caspase-3 activity. The A549 and H460 cells were incubated with TSA (50 ng/ml) alone or combined with genistein (10 μM) for 24h. The ABC-1 cells were incubated with TSA (200 ng/ml) alone or combined with genistein (10 μM) for 24h. Values (means±SD) among the same cell line not sharing a common letter are significantly different ($p<0.05$). Two-way analysis was performed to determine the interaction between TSA and genistein. * denotes a significant interaction between TSA and genistein ($p<0.05$). C: control. G: genistein.





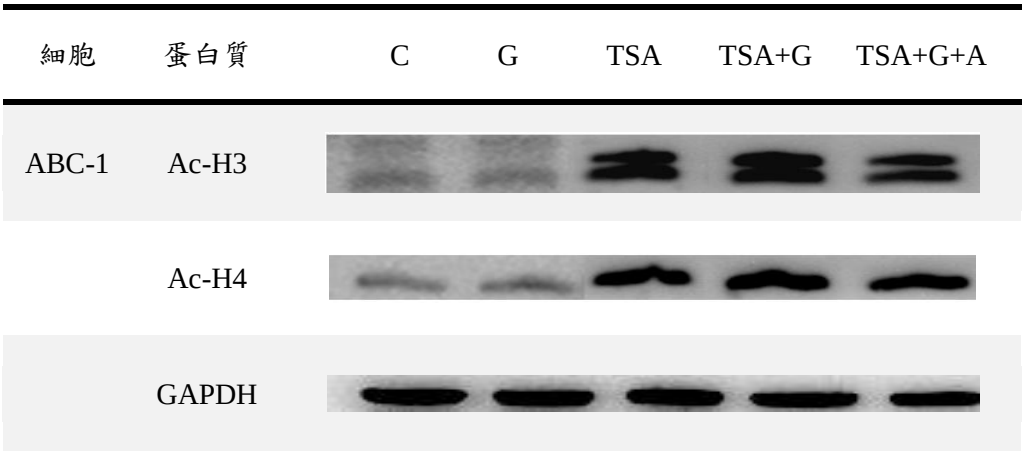


Figure 3、The effects of TSA alone or combined with genistein and/or anacardic acid on the acetylation of histone. The cells were incubated with TSA (50 or 200 ng/ml) alone or combined with genistein (10 μM) and/or anacardic acid (1 μM) for 24h.

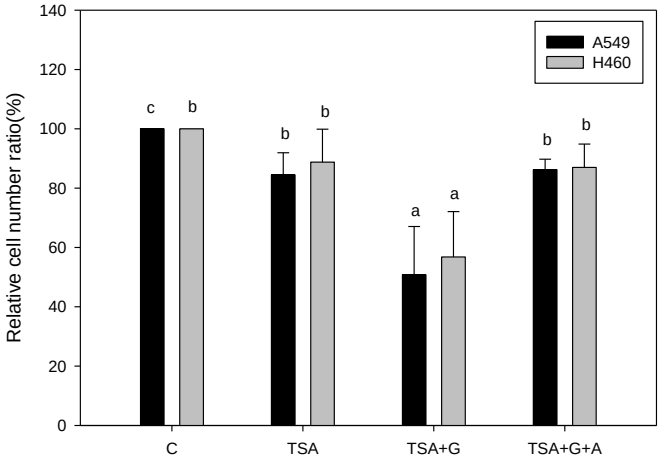


Figure 4、The effect of anacardic acid on cell growth arrest induced by TSA combined with genistein. The cells were incubated with TSA (50 ng/ml) alone or combined with genistein (10 μM) and/or anacardic acid (1μM) for 24h. Values (means±SD) among the same cell line not sharing a common letter are significantly different ($p<0.05$). C: control. G: genistein. A: anacardic acid.

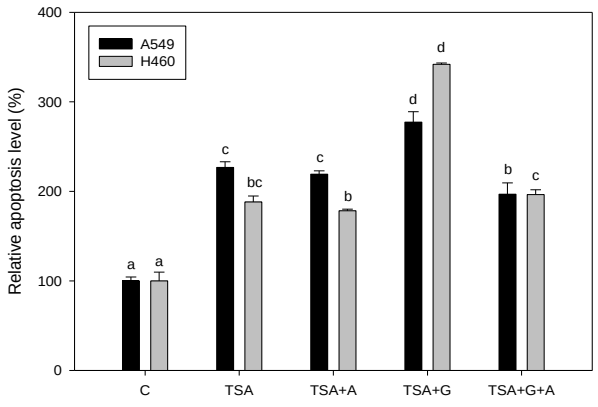


Figure 5、The effect of anacardic acid on cell apoptosis induced by TSA combined with genistein. The cells were incubated with TSA (50 ng/ml) alone or combined with genistein (10 μM) and/or anacardic acid (1 μM) for 48h. Values (means±SD) among the same cell line not sharing a common letter are significantly different ($p<0.05$). C: control. G: genistein. A: anacardic acid.

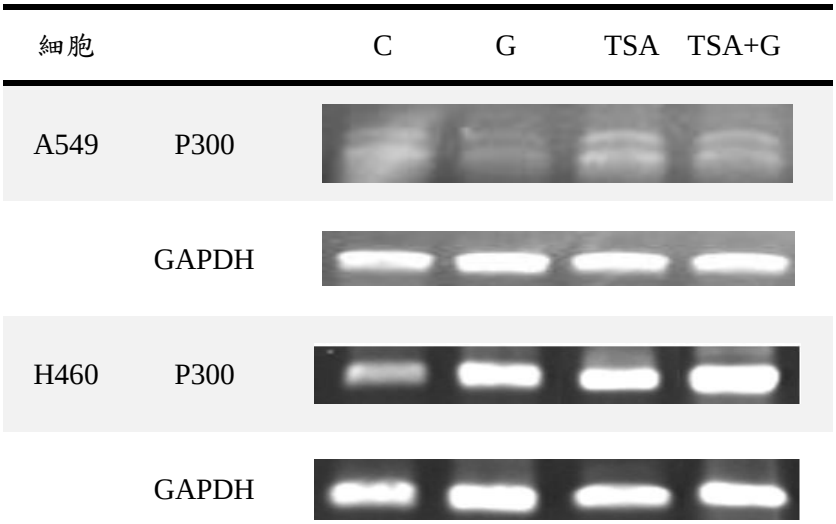


Figure 6.The effects of TSA alone or combined with genistein on p300 mRNA expression in cells. The cells were incubated with TSA (50 ng/ml) alone or combined with genistein (10 μM) for 1h. C: control. G: genistein.

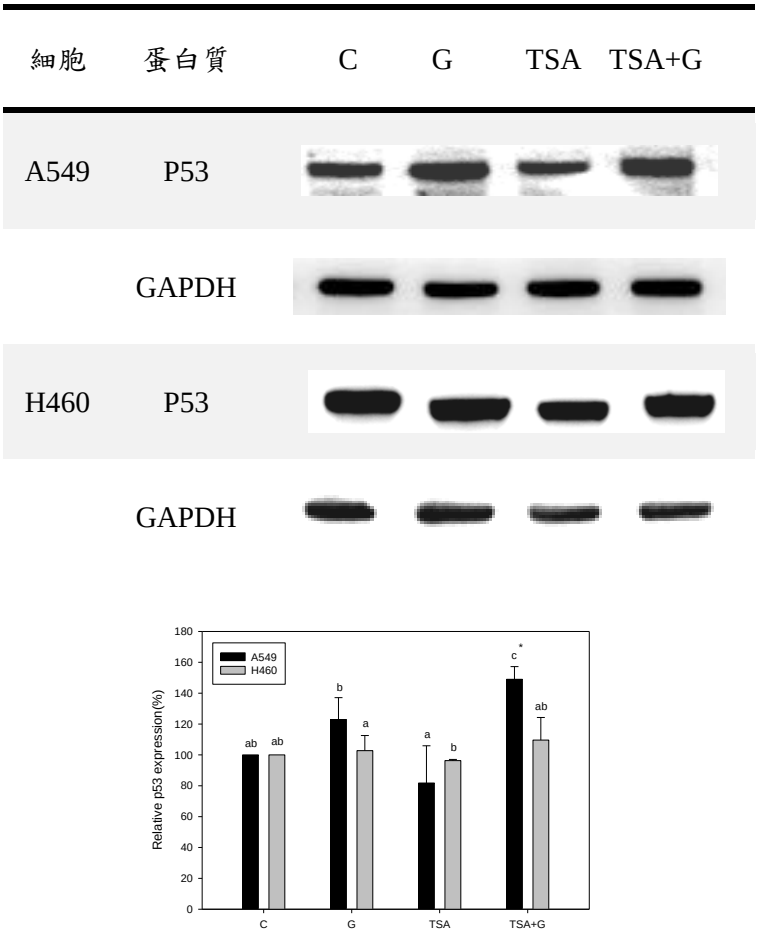
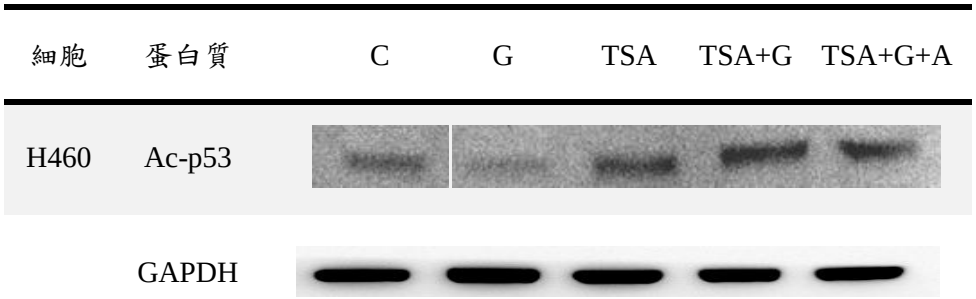


Figure 7.The effects of TSA alone or combined with genistein on p53 protein expression in cells. The cells were incubated with TSA (50 ng/ml) alone or combined with genistein (10 μM) for 12h. Values (means±SD) among the same cell line not sharing a common letter are significantly different ($p<0.05$). Two-way analysis was performed to determine the interaction between TSA and genistein. * denotes a significant interaction between TSA and genistein ($p<0.05$). C: control. G: genistein.



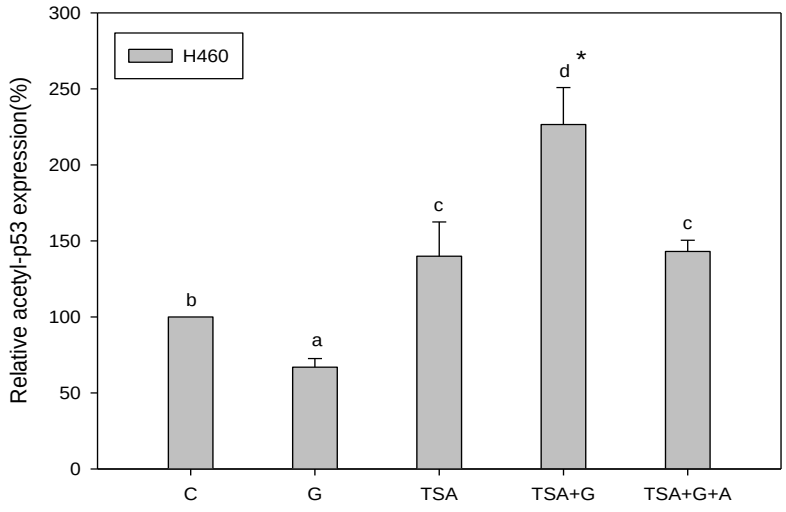


Figure 8.The effects of TSA alone or combined with genistein on acetyl-p53 protein expression in H460 cells. The cells were incubated with TSA (50 ng/ml) alone or combined with genistein (10 μM) for 12h. Values (means±SD) among the same cell line not sharing a common letter are significantly different ($p<0.05$). Two-way analysis was performed to determine the interaction between TSA and genistein. * denotes a significant interaction between TSA and genistein ($p<0.05$). C: control. G: genistein.

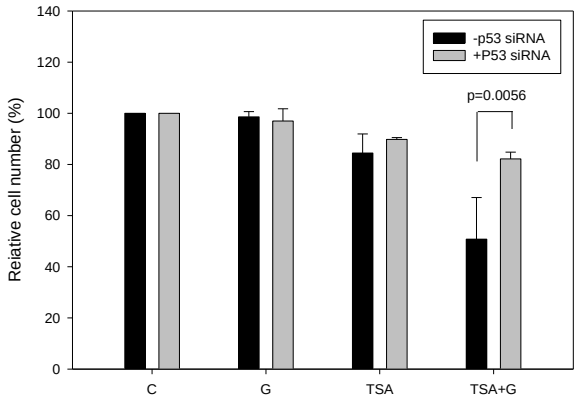


Figure 9.The effects of p53-silencing on the growth of A549 cells exposed to TSA alone or in combination with genistein. The cells were transfected with or without p53 siRNA before incubatation with TSA (50 ng/ml) alone or combined with genistein (10 μM) for 24h. Values (means±SD) between the groups with or without p53 siRNA were compared by student's *t*-test. C: control. G: genistein.

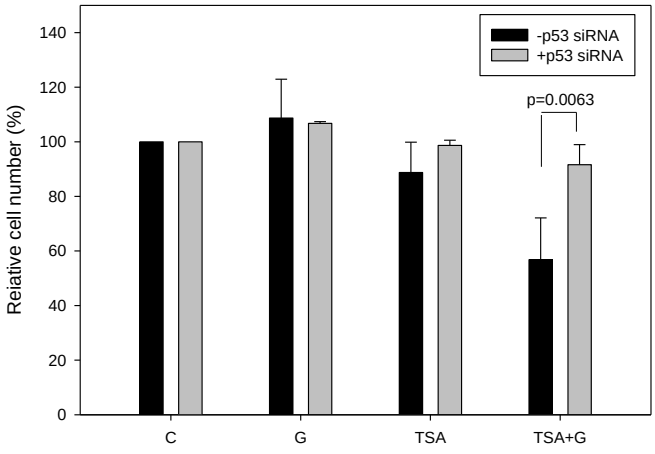


Figure 10.The effects of p53-silencing on the growth of H460 cells exposed to TSA alone or in combination with genistein. The cells were transfected with or without p53 siRNA before incubatation with TSA (50 ng/ml) alone or combined with genistein (10 μM) for 24h. Values (means±SD) between the groups with or without p53 siRNA were compared by student's *t*-test. C: control. G: genistein.

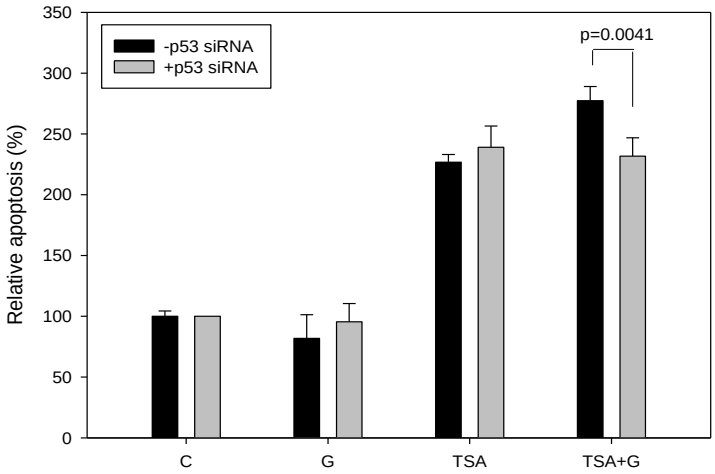


Figure 11、The effects of p53-silencing on the apoptosis of A549 cells exposed to TSA alone or in combination with genistein. The cells were transfected with or without p53 siRNA before incubatation with TSA (50 ng/ml) alone or combined with genistein (10 μM) for 24h. Values (means±SD) between the groups with or without p53 siRNA were compare by student's *t*-test. C: control. G: genistein.

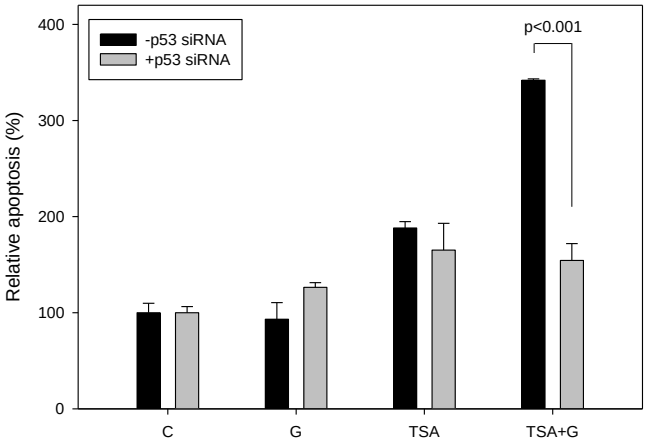


Figure 12、The effects of p53-silencing on the apoptosis of H460 cells exposed to TSA alone or in combination with genistein. The cells were transfected with or without p53 siRNA before incubatation with TSA (50 ng/ml) alone or combined with genistein (10 μM) for 24h. Values (means±SD) between the groups with or without p53 siRNA were compare by student's *t*-test. C: control. G: genistein.

討論

1.以上結果顯示 genistein 促進 TSA 抑制肺癌細胞生長的效果，與細胞對 TSA 之

敏感性有關，對 TSA 敏感的癌細胞，genistein 的增強效果也較佳。

2.genistein 在三株細胞中，皆可以增加 TSA 誘發的組蛋白乙醯化程度，但對誘發細胞凋亡卻有不同的結果。此一差異與 53 蛋白表現有關。

3.將 A549 及 H460 細胞轉染 p53 siRNA，結果發現，於 A549 及 H460 兩株細胞中，當 p53 基因不正常表現時，genistein 促進 TSA 誘發的細胞生長停滯及凋亡現象都消失。

4. genistein 調節組蛋白乙烯化與活化 acetyl transferase 有關 。

第三年(劉上宇、2010)

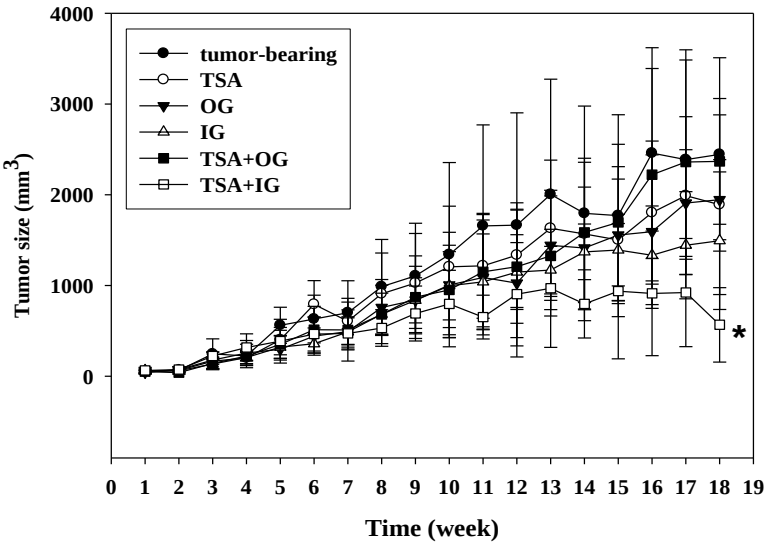


Figure 1. The tumor size of nude mice.After acclimated for 1 week, A549 cells (5×10^6 / 200 μL matrigel solution) were injected into the hind flank region of the nude mice. Three weeks after injection, the nude mice were administered TSA (0.5 mg/kgw) and/or genistein by gavage (OG; 100 mg/kgw) or intraperitoneal injection (IP; 10 mg/kgw) for 14 weeks. The control group was administered with normal-saline solution (the vehicle) only. Values are expressed as mean ± SD (n＝5-7). *denotes significant different from tumor bearing group ($p<0.05$).

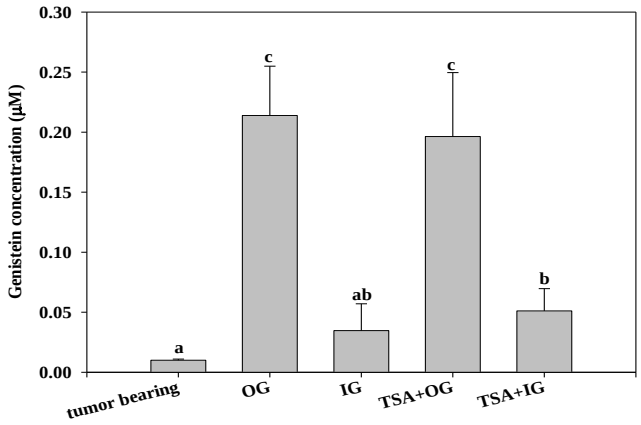


Figure 2. The total concentration of genistein in plasma of nude mice.

After acclimated for 1 week, A549 cells (5×10^6 / 200 μ L matrigel solution) were injected into the hind flank region of the nude mice. Three weeks after injection, the nude mice were administered TSA (0.5 mg/kgw) and/or genistein by gavage (OG; 100 mg/kgw) or intraperitoneal injection (IP; 10 mg/kgw) for 14 weeks. The control group was administered with normal-saline solution (the vehicle) only. Values are expressed as mean ± SD (n＝5-7). Values (mean ± SD, n＝5-7) not sharing a common letter are significantly different ($p< 0.05$).

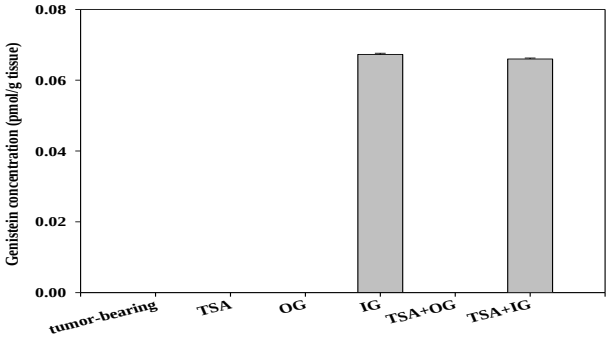


Figure 3. The total concentration of genistein in tumors of nude mice.

After acclimated for 1 week, A549 cells (5×10^6 / 200 μ L matrigel solution) were injected into the hind flank region of the nude mice. Three weeks after injection, the nude mice were administered TSA (0.5 mg/kgw) and/or genistein by gavage (OG; 100 mg/kgw) or intraperitoneal injection (IP; 10 mg/kgw) for 14 weeks. The control group was administered with normal-saline solution (the vehicle) only. Values are expressed as mean ± SD (n＝5-7). Values (mean ± SD, n＝5-7) not sharing a common letter are significantly different ($p< 0.05$).

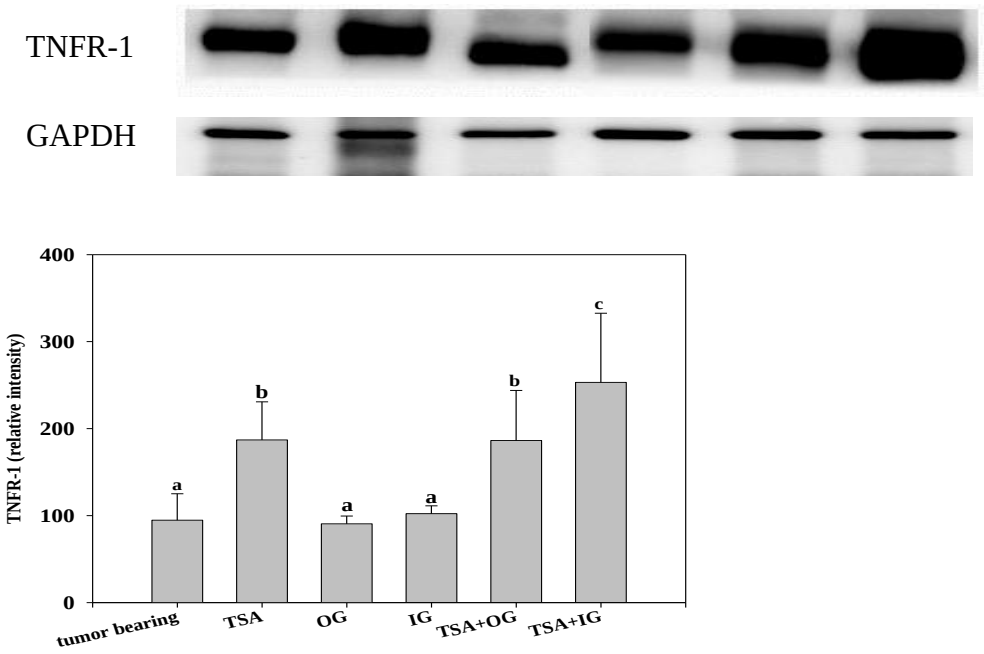
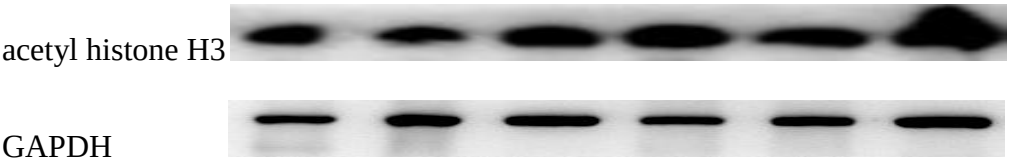
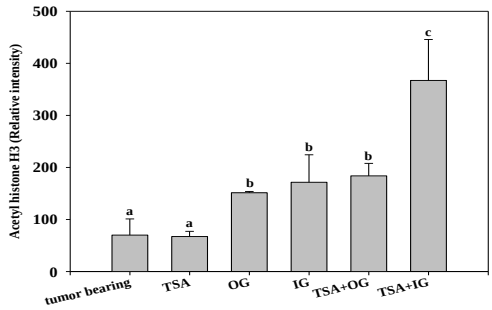
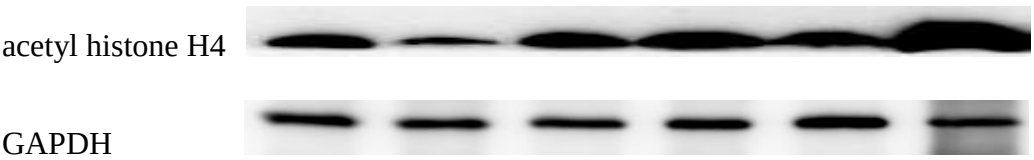


Figure 4. The effect of TSA alone or in combination with genistein on the expression of TNFR-1 protein in tumors.

After acclimated for 1 week, A549 cells (5×10^6 / 200 μ L matrigel solution) were injected into the hind flank region of the nude mice. Three weeks after injection, the nude mice were administered TSA (0.5 mg/kgw) and/or genistein by gavage (OG; 100 mg/kgw) or intraperitoneal injection (IP; 10 mg/kgw) for 14 weeks. The control group was administered with normal-saline solution (the vehicle) only. Values are expressed as mean ± SD (n＝5-7). Values (mean ± SD, n＝5-7) not sharing a common letter are significantly different ($p< 0.05$).







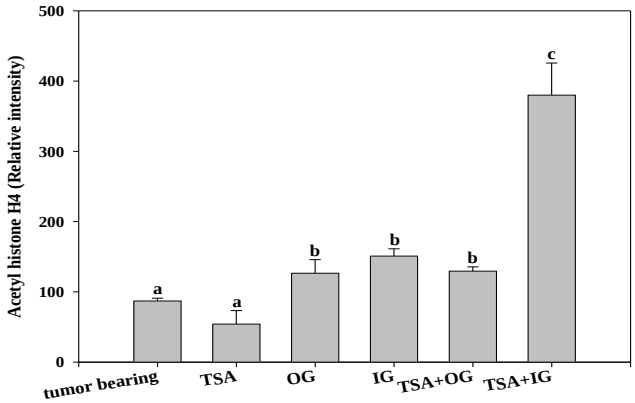


Figure 5. The effect of TSA alone or in combination with genistein on the expression of acetyl histone H3 and H4 protein in tumors. After acclimated for 1 week, A549 cells (5×10^6 / 200 μ L matrigel solution) were injected into the hind flank region of the nude mice. Three weeks after injection, the nude mice were administered TSA (0.5 mg/kgw) and/or genistein by gavage (OG; 100 mg/kgw) or intraperitoneal injection (IP; 10 mg/kgw) for 14 weeks. The control group was administered with normal-saline solution (the vehicle) only. Values are expressed as mean ± SD (n＝5-7). Values (mean ± SD, n＝5-7) not sharing a common letter are significantly different ($p< 0.05$).

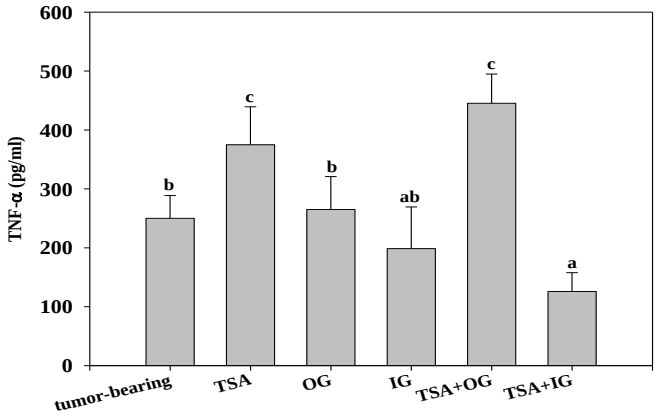


Figure 6.The level of TNF-α in plasma of nude mice.

After acclimated for 1 week, A549 cells (5×10^6 / 200 μL matrigel solution) were injected into the hind flank region of the nude mice. Three weeks after injection, the nude mice were administered TSA (0.5 mg/kgw) and/or genistein by gavage (OG; 100 mg/kgw) or intraperitoneal injection (IP; 10 mg/kgw) for 14 weeks. The control group was administered with normal-saline solution (the vehicle) only. Values are expressed as mean ± SD (n＝5-7). Values (mean ± SD, n＝5-7) not sharing a common letter are significantly different ($p< 0.05$).

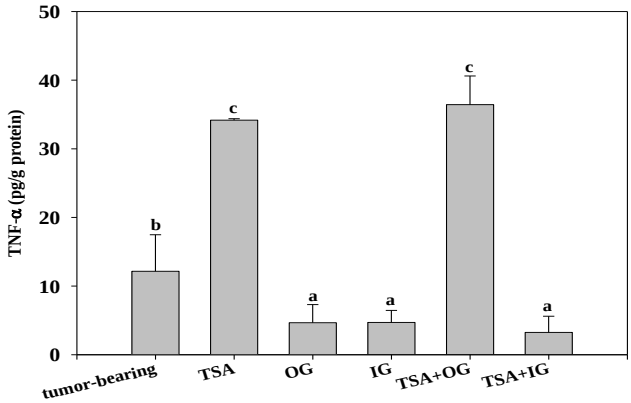


Figure 7.The level of TNF-α in tumors of nude mice.

After acclimated for 1 week, A549 cells (5×10^6 / 200 μL matrigel solution) were injected into the hind flank region of the nude mice. Three weeks after injection, the nude mice were administered TSA (0.5 mg/kgw) and/or genistein by gavage (OG; 100 mg/kgw) or intraperitoneal injection (IP; 10 mg/kgw) for 14 weeks. The control group was administered with normal-saline solution (the vehicle) only. Values are expressed as mean ± SD (n＝5-7). Values (mean ± SD, n＝5-7) not sharing a common letter are significantly different ($p< 0.05$).

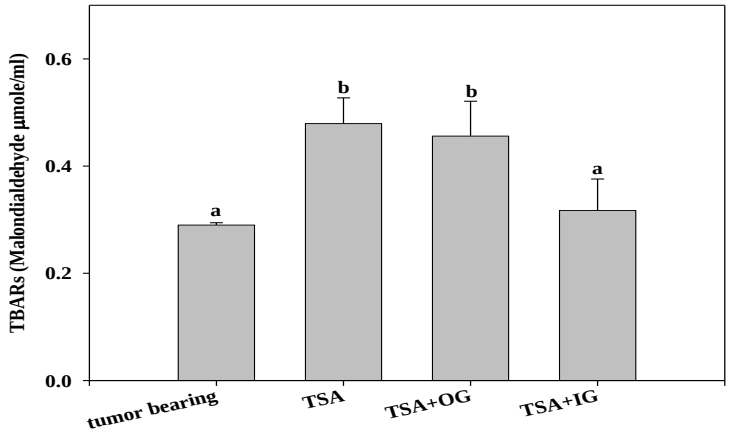


Figure 8. Effects of TSA administration at 0.5 mg/kgw alone or in combination with genistein at 10 or 100 mg/kgw (IG and OG) on the levels of TBARs in plasma. Values (mean ± SD, n=5-7) not sharing a common letter are significantly different ($p < 0.05$).

討論

1.研究結果發現裸鼠經腹腔注射 genistein 合併 TSA 處理，與 tumor bearing 組相比，能顯著降低 tumor size，但 TSA 或 IG 本身則無顯著效應，此一結果應證我們在體外觀察的結果 (Yang , 2010)，顯示 genistein 在體內可以增強 TSA 的抗癌效果，且此合併處理不會影響小鼠體重。

2. 我們亦比較管餵及注射 genistein 對促進 TSA 抗癌效果的差異，結果發現管餵 genistein，雖然其劑量是注射組的 10 倍，卻沒有增加 TSA 作用的效果，此一結果可能與 genistein 在體內的形式及分佈有關。

3. 研究中觀察各組間腫瘤組織中組蛋白 H3、H4 之乙醯基化表現的差異，與 TNFR-1 蛋白表現相似，TSA 合併腹腔注射 genistein 處理，較 TSA 單獨處理顯著增加組蛋白 H3、H4 乙醯基化效果及 TNFR-1 蛋白表現 (p <0.05)，此結果亦與我們的體外研究相似（Chen, 2007）。

4.化療可能會引起 tumor necrosis factor-α (TNF-α)或 interleukin-1 等細胞激素的增加，引起局部發炎反應稱家氧化壓力，我們的研究顯示注射 genistein 可以降低 TSA 誘發的 TNF-α 及 TBARS。

五、結論

綜合以上結果，本研究證實TSA與genistein在體內具交互作用，genistein可增加TSA之抗腫瘤的效果但其效果視腫瘤p53蛋白影響，往後需進一步證實這策略的臨床應用上之可能性。

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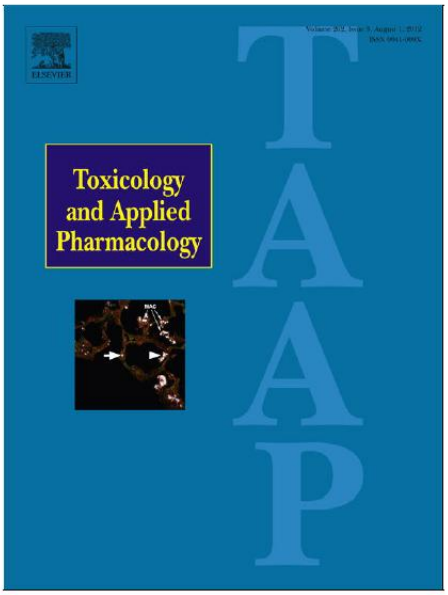

Toxicology and Applied Pharmacology 262 (2012) 247–254

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Toxicology and Applied Pharmacology

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Genistein enhances the effect of trichostatin A on inhibition of A549 cell growth by increasing expression of TNF receptor-1☆

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ARTICLE INFO

Article history:
Received 7 February 2012
Revised 27 April 2012
Accepted 2 May 2012
Available online 14 May 2012

Keywords:
Genistein
Trichostatin A
TNF receptor-1
A549 cells

ABSTRACT

Our previous study has shown that genistein enhances apoptosis in A549 lung cancer cells induced by trichostatin A (TSA). The precise molecular mechanism underlying the effect of genistein, however, remains unclear. In the present study, we investigated whether genistein enhances the anti-cancer effect of TSA through up-regulation of TNF receptor-1 (TNFR-1) death receptor signaling. We incubated A549 cells with TSA (50 ng/mL) alone or in combination with genistein and then determined the mRNA and protein expression of TNFR-1 as well as the activation of downstream caspases. Genistein at 5 and 10 μM significantly enhanced the TSA-induced decrease in cell number and apoptosis in a dose-dependent manner. The combined treatment significantly increased mRNA and protein expression of TNFR-1 at 6 and 12 h, respectively, compared with that of the control group; while TSA alone had no effect. TSA in combination with 10 μM of genistein increased TNFR-1 mRNA and protein expression by about 70% and 40%, respectively. The underlying mechanism for this effect of genistein may be partly associated with the estrogen receptor pathway. The combined treatment also increased the activation of caspase-3 and -10 as well as p53 protein expression in A549 cells. The enhancing effects of genistein on the TSA-induced decrease in cell number and on the expression of caspase-3 in A549 cells were suppressed by silencing TNFR-1 expression. These data demonstrated that the upregulation of TNFR-1 death receptor signaling plays an important role, at least in part, in the enhancing effect of genistein on TSA-induced apoptosis in A549 cells.

Introduction

Lung cancer is the leading cause of cancer death in many countries. However, the treatment success rate of this disease remains low (Lin et al., 2010). Trichostatin A (TSA), a novel anticancer drug, is a histone deacetylase inhibitor which increases the accumulation of acetylated forms of histones and nonhistone proteins and leads to transcription of cell-growth-arrest and apoptosis associated genes (Kim et al., 2006). Studies have shown that TSA may be a potential therapy for lung cancer because it induces apoptosis of lung cancer cells by activating the death receptor and mitochondrial-mediated pathway (Dokmanovic and Marks, 2005; Kim et al., 2006). However, the toxicity of this drug limits its application (Lane and Chabner, 2009). For example, TSA enhances hypertrophy in cardiac myocytes induced by doxorubicin, a conventional anticancer drug (Karagiannis et al., 2010).

Genistein is an isoflavone present in soybeans and soybean products. Growing evidence shows that dietary isoflavones including genistein may protect against several cancers including lung cancer (Gadgeel et al., 2009; Li and Chen, 2011; Nagata et al., 2007). Epidemiologic studies also show that the intake or the plasma level of genistein is associated with a decreased risk of lung cancer (Schabath et al., 2005; Shimazu et al., 2011). The possible mechanisms by which genistein influences the growth of cancer cells are various, including inhibition of protein–tyrosine kinase, activation of p53 and p21 and regulation of cell signaling transduction pathways (Sarkar et al., 2010; Seo et al., 2011). In addition, several studies have shown how genistein may cooperatively or synergistically enhance the effect of anticancer drugs to inhibit the proliferation of cancer cells (Ali et al., 2009; Gadgeel et al., 2009; Lattrich et al., 2011).

Combination therapy has attracted more attention recently because it may reduce the toxicity of chemotherapy due to the lower dose of each compound (Ali et al., 2009; Ganslmayer et al., 2004;

Abbreviations: DMSO, Dimethyl sulfoxide; RT-PCR, Reverse transcription PCR; siRNA, small interference RNA; TNFR-1, TNF receptor-1; TSA, Trichostatin A.

☆ **Grant:** This research was supported by grants (NSC-96-2320-B040-029-MY3) from the National Science Council, Republic of China.

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doi:10.1016/j.taap.2012.05.003

國科會補助計畫衍生研發成果推廣資料表

日期:2013/11/07

國科會補助計畫	計畫名稱: Genistein促進trichostatin A抑制人類非小細胞肺癌細胞生長的體外及體內研究
	計畫主持人: 葉姝蘭
	計畫編號: 99-2320-B-040-006-MY3　　學門領域: 保健營養

無研發成果推廣資料

99 年度專題研究計畫研究成果彙整表

計畫主持人：葉姝蘭	計畫編號：99-2320-B-040-006-MY3
計畫名稱：Genistein 促進 trichostatin A 抑制人類非小細胞肺癌細胞生長的體外及體內研究	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	3	3	100%		
		研討會論文	3	3	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	3	3	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	1	2	100%	篇	Wu TC , Yang YC, Huang PR, Wen YD, Yeh SL* Genistein enhances the effect of trichostatin A on inhibition of A549 cell growth by increasing expression of TNF receptor-1. Toxicology and Applied Pharmacology. 2012, 262 : 247-254 (SCI)（IF = 4.447 ; R/C=10/83 , TOXICOLOGY）
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	

		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
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		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)		有助臨床實用性					

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：■已發表 □未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

1.已發表一篇 paperWu TC , Yang YC, Huang PR, Wen YD, Yeh SL* Genistein enhances the effect of trichostatin A on inhibition of A549 cell growth by increasing expression of TNF receptor-1. Toxicology and Applied Pharmacology. 2012, 262：247-254(SCI) （IF＝4.447；R/C=10/83）

2.另有文稿準備發表中

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限）

1.找出 Genistein 增強 TSA 誘發肺癌 A549 細胞凋亡的分子途徑:Toxicology and Applied Pharmacology. 2012, 262：247-254

2.利用四株不同的肺癌細胞，本研究結果顯示在不同的肺癌細胞中，Genistein 促進 TSA 的抗腫瘤效果與細胞對 TSA 敏感性有關。透過增加組蛋白乙醯轉移酶活性，Genistein 增加 TSA 誘發的組蛋白乙醯化程度，進而誘發細胞生長停滯及細胞凋亡。然而，下游蛋白的表現，例如腫瘤抑制因子-p53，可能會影響 TSA 單獨或合併 genistein 的抗腫瘤之效果。

3.利用動物易體移植模式，我們證實皮下注射 Genistein 可以顯著增加 TSA 抑制腫瘤增生的效果且其機制與增加 Histone acetylation 及 TNFR-1 表現有關。

本研究首先證實了 genistein 體內外增強 TSA 抗腫瘤的效果，並研究出其部分的分子機制，對未來的臨床實用性具有良好的參考價值。