科技部補助專題研究計畫成果報告
期末報告

槲皮酮對組蛋白去乙醯酶抑制劑抗腫瘤功效的影響：體外及體內研究(第2年)

計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： NSC 102-2320-B-040-011-MY2
執 行 期 間 ： 103年08月01日至104年07月31日
執 行 單 位 ： 中山醫學大學營養學系（所）

計 畫 主 持 人： 葉姝蘭

計畫參與人員： 碩士班研究生-兼任助理人員：陳韻竹
碩士班研究生-兼任助理人員：莊喻荏
博士班研究生-兼任助理人員：詹淑婷

處 理 方 式 ：
1.公開資訊：本計畫涉及專利或其他智慧財產權，2年後可公開查詢
2.「本研究」是否已有嚴重損及公共利益之發現：否
3.「本報告」是否建議提供政府單位施政參考：否

中 華 民 國 104 年 10 月 15 日

中 文 摘 要 ： 在先前的研究中，我們發現在A549或H1299細胞中，quercetin透過非p53依賴的機制增加TSA所誘發的組蛋白H3及H4的乙醯化作用，然而，其確切機制並不清楚。因此我們首先探討在H1299細胞中，quercetin增強TSA（或vorinostat，另一種組蛋白去乙醯酶抑制劑）增加組蛋白H3及H4乙醯化對其促進細胞凋亡的角色。Quercetin藉由增加DR5（一種死亡接受器）的表現而增強TSA造成H1299細胞的凋亡，quercetin合併TSA會顯著的增加p300蛋白（組蛋白乙醯轉移酶）的表現，但TSA本身則否，而將H1299細胞中的p300 RNA silencing後，顯著的消減quercetin增強TSA誘發的組蛋白H3及H4乙醯化、DR5蛋白的表現及細胞凋亡的情形。在H1299細胞當中quercetin合併vorinostat的效果及機制與quercetin合併TSA是相似的。

第二年，我們探討quercetin經由管餵（20 and 100 mg/kg body weight，每周三次）方式是否也會增加TSA抗腫瘤的效果。我們發現quercetin以這樣的劑量經由管餵方式給予無法增加TSA的抗癌效果，這可能與quercetin的代謝轉換有關，體外實驗證明，quercetin的代謝產物quercetin-3-glucuronide增加TSA抗腫瘤作用的效果較quercetin差，且其併入細胞的效果亦較差。然而，quercetin經由管餵或是腹腔注射方式給予腫瘤小鼠皆能降低TSA所誘發淋巴細胞DNA的傷害及血漿中脂質過氧化的情形。

綜合以上，此二年期計畫證明quercetin向上調節p300繼而調節histone乙醯化，是quercetin增強histone去乙醯酶抑制劑抗腫瘤效果的重要機制。Quercetin以口服方式增強的效果較腹腔注射方式效果差，這與quercetin代謝產物的轉換有關。然而，不論是以口服或腹腔注射方式給予quercetin皆能降低TSA所誘發淋巴細胞DNA的傷害及脂質過氧化的情形。

中 文 關 鍵 詞 ： quercetin、trichostatin A、 quercetin代謝產物、p300

英 文 摘 要 ： In our previous study, we found that quercetin increased the acetylation of histones H3 and H4, which may modulate apoptosis-associated genes expression, induced by TSA in both A549 and H1299 cells through p53-independent mechanisms. However, the precise mechanisms are unclear. Thus, we first investigated the role of increasing acetylation of histones H3 and H4 by quercetin in the apoptosis in H1299 cells exposed to TSA (or vorinostat, another histone deacetylase inhibitor). Quercetin enhanced TSA-induced apoptosis in H1299 cells through increasing DR5, a death receptor, expression. Quercetin+TSA rather than TSA alone also significantly increased p300, a histone acetyltransferase, expression. Transfection of p300 siRNA significantly diminished the increase of histones H3/H4 acetylation, DR5 protein expression, and apoptosis induced by quercetin in H1299 cells exposed to TSA. The combined effects and the mechanisms of quercetin+vorinostat on the apoptosis in H1299 cells were similar to those of quercetin+TSA.

Furthermore, we investigated whether quercetin administered by gavage (20 and 100 mg/kg body weight, 3 times/week) enhances the antitumor effect of TSA. We found that quercetin given orally at these doses failed to enhance the antitumor effect of TSA because of its metabolic conversion. The in vitro study demonstrated that the enhancing effect and the incorporation of quercetin metabolite, quercetin-3-glucuronide, was less than that of quercetin in A549 cells exposed to TSA. However, oral and intraperitoneal administration of quercetin similarly decreased TSA-induced lymphocyte DNA damage and plasma lipid peroxidation in tumor-bearing mice.
In conclusion, this two year research demonstrates that the up-regulation of p300 expression, which in turn increases histone acetylation, by quercetin plays an important role in enhancing histone deacetylase inhibitor-induced apoptosis in H1299 cells. In vivo, the enhancing effect of quercetin administration orally on the antitumor effect of TSA is poor than that of intraperitoneal administration because of metabolic conversion. However, quercetin administrated both orally and intraperitoneally similarly decreases TSA-induced lymphocyte DNA damage and lipid peroxidation.

英文關鍵詞：quercetin、trichostatin A、 quercetin metabolite、p300

1. Introduction

Quercetin, a flavonoid, is ubiquitously found in foods such as lettuce, chili peppers, cranberries, onions, tomatoes, broccoli and apples [García-Mediavilla et al., 2007; Bureau et al., 2008; Murakami et al., 2008]. Growing evidences show that quercetin may cooperatively or synergistically enhance the anticancer effects of chemotherapy drugs. In our previous study we found that quercetin at 2-5 μM synergistically enhanced the effects of trichostatin A (TSA), a histone deacetylase inhibitor (HDI) on suppressing proliferation and inducing apoptosis in human lung carcinoma through p53-dependent pathway in vitro and in vivo. Our study also found that quercetin increased the levels of acetylated histones H3/H4 in lung cancer cells in a p53-independent manner [Chan et al., 2013]. However, whether this related to the p53-independent enhancing effect of quercetin on the antitumor effect of TSA is unclear. In addition, it has been shown that after quercetin intake, conjugated metabolites, such as quercetin glucuronides and quercetin sulfates, rather than quercetin aglycone are prevalent in human and animal plasma [Kim et al., 2006; Dokmanovic et al., 2005; Seo et al., 2011]. The bioactivities among quercetin and its metabolites could be different because of structure modification [Lee et al., 2011]. Thus, in the two-year study we first investigated the possible mechanisms and the role of the acetylation of histones H3 and H4 increased by quercetin in the apoptosis of H1299 cells (a p53 mutant human lung cancer line) exposed to TSA (or vorinostat). Second, we investigated the effect of oral administration of quercetin on the antitumor effect and toxic effect of TSA in tumor-bearing mice, compared with intraperitoneal administration. We also used A549 cells to compare the intracellular accumulation and the enhancing effect of quercetin and quercetin-3-gluquronide (Q3G) on the anti-proliferation effect of TSA.

2. Methods

The first year

H1299 cells were incubated with TSA/vorinostat alone or in combination with quercetin. We first used an oligonucleotide array assay to determine the possible pathway involved in the enhancing effect of quercetin on the antitumor effect of TSA. We found that compared with TSA alone quercetin increased the expression of the death receptor 5 (DR5), which is well known to mediate extrinsic apoptosis pathways, [Chen et al., 2007; Jung et al., 2010]. Therefore, we further determined the mRNA and protein expression of DR5 as well as the downstream caspases in H1299 cells with various treatments. Second, using an inhibitor of histone acetyl transferase we investigated the role of acetylation of histones H3 and H4 increased by quercetin in H1299 cells exposed to TSA and the possible mechanism.

The second year

Male nude mice aged 5 to 6 weeks were subcutaneously injected in the right flank with A549 cells at a dose of 5×10^6 cells (in 200 μL of matrigel). Three weeks after cell injection, the animals were then randomly assigned to the following six groups (n=6/group) for 16 weeks: control, TSA, OL+TSA, OH+TSA, IL+TSA and IH+TSA for TSA alone or in combination with quercetin treatment. TSA was given twice a week (0.5 mg/kg body weight) by intraperitoneal (i.p.) injection, while quercetin was given 3 times a week by oral gavage (OL and OH, 20 and 100 mg/kg body weight, respectively) or by i.p. injection (IL and IH, 2 and 10 mg/kg body weight, respectively). During the experimental period, the body weights of the mice were recorded weekly. Blood samples were collected at weeks 4-10 (for determining quercetin concentration) and at week 17 (for determining lipid peroxidation level) from the retro-orbital plexus of the nude mice under deep isoflurane anesthesia. After

the experiment, the animals were sacrificed. Blood and tissue samples were collected to determine the level of quercetin, quercetin metabolites, and were stored at -80℃ until analyzed.

In addition, we also incubated A549 cells with TSA alone or in combination with quercetin or quercetin-3-gluquronide (Q3G) to compare the intracellular accumulation and the enhancing effect of quercetin and Q3G on the anti-proliferation effect of TSA.

3. Results & Discussion

The first year

Part of this article has published in :
Shu-Ting Chan. Studies of quercetin on the antitumor and the side effects of trichostatin A. Doctoral Dissertation, 2015.

The results showed that quercetin (5 μM) significantly increased the apoptosis in H1299 cells induced by 25 ng/mL (82.5 nM) of TSA at 72 h by 88% (Fig. 1). Quercetin also significantly increased TSA-induced the death receptor 5 (DR5) mRNA and protein expression as well as caspase-10/3 activities in H1299 cells (Fig. 2). Transfection of *DR5* siRNA into H1299 cells significantly diminished the enhancing effects of quercetin on TSA-induced apoptosis (Fig. 3). These results demonstrated that quercetin enhanced the apoptosis induced by TSA in H1299 cells (p53 null mutant) by increasing the expression of DR5 through a p53-independent mechanism. It has been shown that quercetin sensitizes TRAIL-induced apoptosis in non-small cell lung cancer through PKC-mediated DR5 induction [Chen et al., 2007] Furthermore, TSA in combination with quercetin rather than TSA alone significantly increased p300 (a histone acetyltransferase) expression (Fig. 4). Transfection of *p300* siRNA significantly diminished the increase of histones H3/H4 acetylation, DR5 protein expression, caspase-10/3 activity and apoptosis induced by quercetin in

H1299 cells exposed to TSA (Fig. 6). In addition, quercetin also similarly and significantly increased vorinostat-induced cell-growth-arrest and apoptosis through the p300-dependent pathway because transfection of *p300* siRNA significantly diminished the enhancing effects of quercetin (Fig. 7, 8). These data indicate that the up-regulation of p300 expression, which in turn increases histone acetylation, by quercetin plays an important role in enhancing histone deacetylase inhibitor-induced apoptosis in H1299 cells.

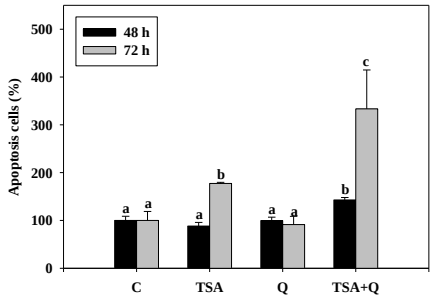


Figure 1. Effects of trichostatin A (TSA) alone or in combination with quercetin (Q) on the apoptosis of H1299 cells.

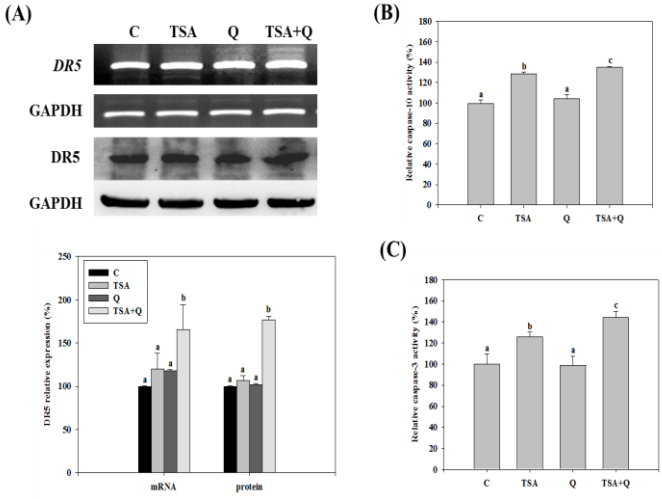


Figure 2. Effects of trichostatin A (TSA) alone or in combination with quercetin (Q) on the *death receptor 5* (*DR5*) mRNA and protein expression (A), as well as caspase-10 (B) and caspase-3 activities (C) in H1299 cells.

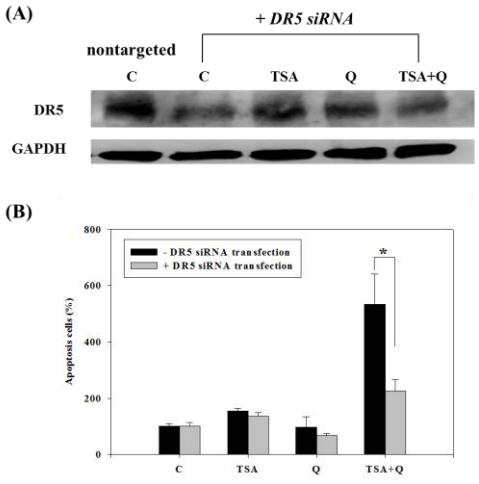


Figure 3. Effects of *death receptor 5* (*DR5*) siRNA transfection on the expression of DR5 protein (A) and apoptosis (B) in H1299 cells.

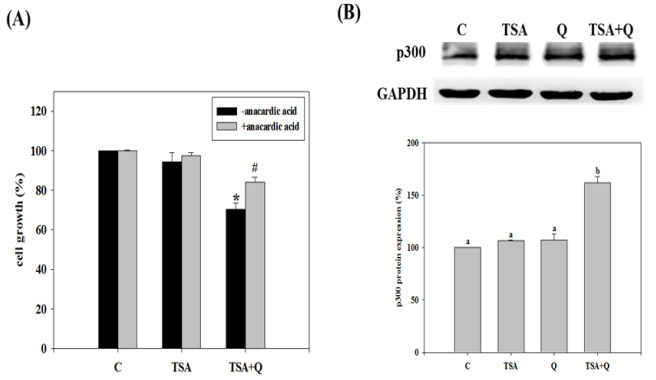


Figure 4. Cell-growth-arrest (A) in H1299 cells with or without anacardic acid and the expression of p300 (B) in cells with various treatments.

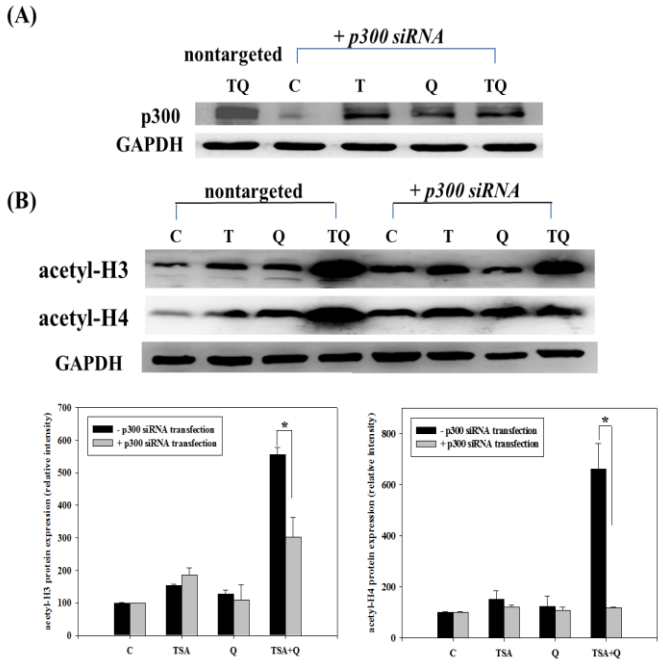


Figure 5. Effects of *p300* siRNA transfection on p300 protein expression (A) and the expression of acetyl histones H3 (acetyl-H3) and H4 (acetyl-H4) (B) in H1299 cells.

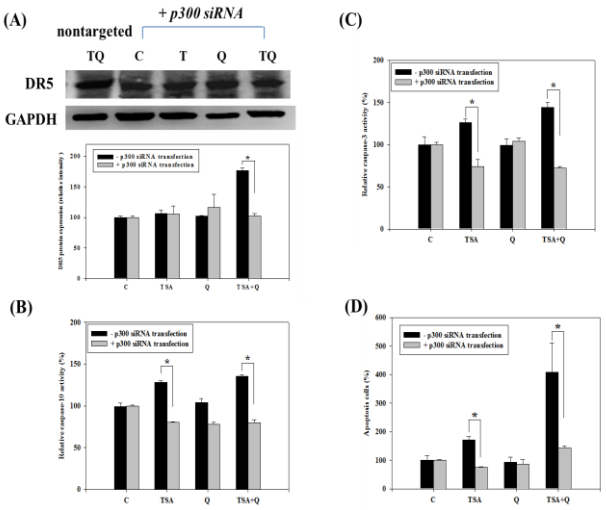


Figure 6. Effects of *p300* siRNA transfection on death receptor 5 (DR5) protein expression (A), the activity of caspase-10 (B), caspase-3 (C) and apoptosis (D) in H1299 cells.

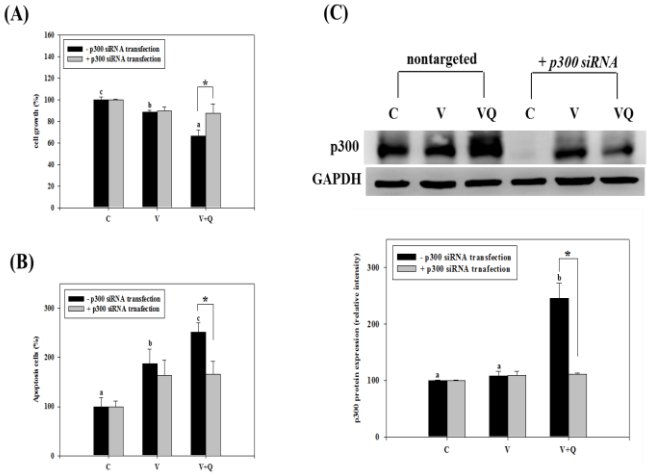


Figure 7. Effects of vorinostat (V) alone or in combination with quercetin (Q) in H1299 cells with or without *p300*-silencing on cell growth (A), apoptosis (B), and p300 protein expression (C)

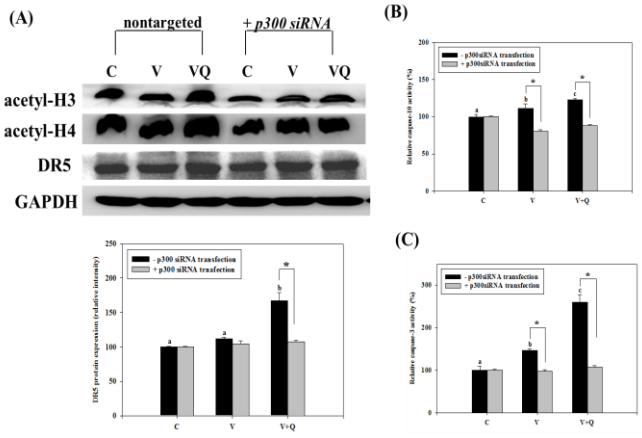


Figure 8. Effects of vorinostat (V) alone or in combination with quercetin (Q) in H1299 cells with or without *p300*-silencing on the expression of acetyl histones H3 (acetyl-H3)/H4 (acetyl-H4) and death receptor 5 (DR5) protein (A) as well as caspase-10 (B) and caspase-3 activity (C) in H1299 cells.

The second year

Part of this article has published in :

BioMed Research International (Journal of Biomedicine and Biotechnology), 2014:580626.

Quercetin given orally (20 and 100 mg/kg body weight, 3 times/week) failed to enhance the antitumor effect of TSA (Fig. 1) although it increased the total quercetin concentration more than quercetin administered intraperitoneally in the plasma (Fig. 2). The compound quercetin-3-glucuronoide (Q3G) increased the most (Fig. 3). However, quercetin administered intraperitoneally increased the total quercetin level in tumor tissues more than oral quercetin (Fig. 3). There data suggest that the overall enhancing effects of quercetin metabolites on the antitumor effect of TSA are less than quercetin itself in vivo. It has been shown that chemopreventive compounds including phytochemicals may have different biological effects between oral administration and intraperitoneal administration because the absorbance and metabolism of these compounds affect their concentrations and structures in vivo [26, 27]. However, oral and intraperitoneal administration of quercetin similarly decreased lymphocyte DNA damage and plasma lipid peroxidation level induced by TSA (Fig.4). These findings suggest that quercetin, even oral administration, diminishes the oxidative stress induced by chemotherapy drugs in normal cells. In further cell study, we found that the enhancing effect of Q3G on the antitumor effect of TSA and the incorporation of Q3G was less than that of quercetin in A549 cells (Fig. 5). The accumulation of Q3G in A549 cells was also less than quercetin (Table1). These data provide the explanation about the poor enhancing efficiency of oral quercetin administration on the antitumor effect of TSA. However, the in vitro study showed that A549 cells possessed the ability to converted Q3G to quercetin. In conclusion, the

present study showed that the oral administration of quercetin at doses of 20 and 100 mg/kg body weight, 3 times per week failed to enhance the antitumor effect of TSA. This result was associated with the metabolic conversion of quercetin in vivo. However, quercetin administered orally diminished TSA-induced adverse effects in nude mice and the effect was similar to that of i.p. injections.

Table 1
Accumulation of quercetin (Q) and quercetin-3-glucuronide (Q3G) in A549 cells.

	Compound	
	Q3G (n mole/g protein)	**Q (n mole/g protein)**
Group	**1 h**	
C	**1.39 ± 0.01** [a]	**0.70 ± 0.35** [a]
Q	**1.40 ± 0.01** [a]	**2.36 ± 0.69** [b]
Q3G	**1.99 ± 0.03** [b]	**0.77 ± 0.21** [a]
	4 h	
C	**1.39 ± 0.01** [a]	**0.70 ± 0.35** [a]
Q	**1.88 ± 0.09** [b]	**10.70 ± 1.14** [c]
Q3G	**2.48 ± 0.01** [c]	**2.97 ± 0.10** [b]

The cells were incubated with 5 μM quercetin Q or Q3G for 1 h and 4 h. Values (means ± SD, n=3) of the same compound and the same time point not sharing a common letter are significantly different (p<0.05).

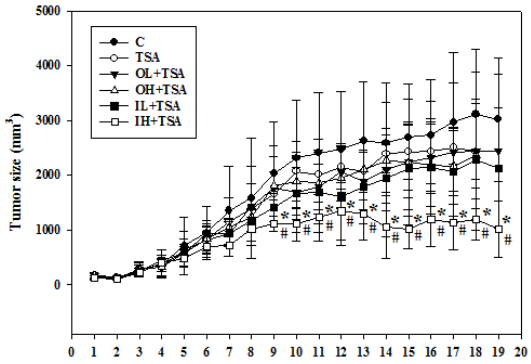


Figure 1. Effects of trichostatin A (TSA) alone or in combination with quercetin on tumor growth in tumor-bearing mice. Values (means ± SD, n=6) with a * denote a significant different from the control group, while a # is significantly different from the TSA group (p<0.05).

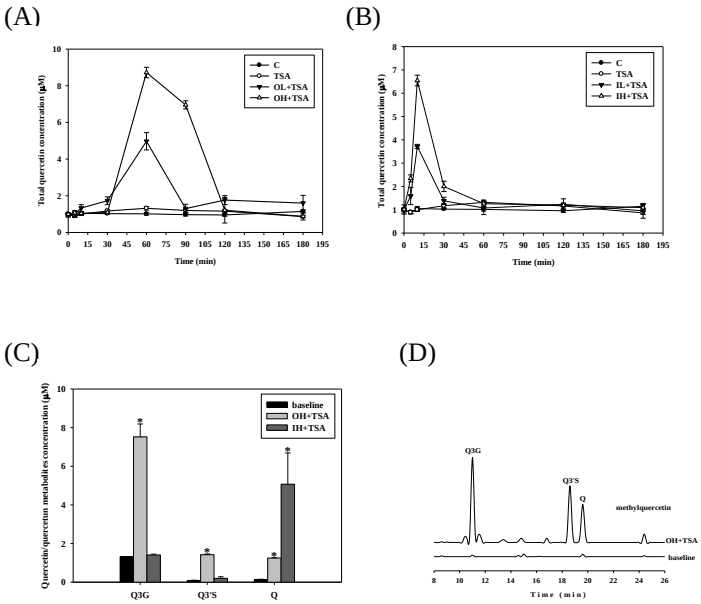


Figure 2. Total (A and B) and individual (C) concentrations of quercetin (Q) and its metabolites in plasma of tumor-bearing mice with various treatments as well as the HPLC chromatograms in the plasma (D). Values are expressed as means ± SD (n=6) and those of the same compound not sharing a common letter are significantly different (p<0.05).

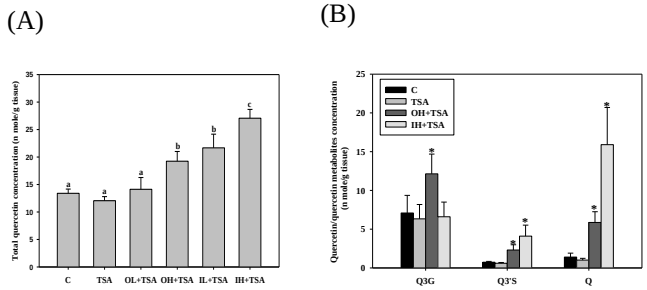


Figure 3. Total (A) and individual (B) concentrations of quercetin (Q) and its metabolites in tumor tissues of tumor-bearing mice with various treatments. Values are expressed as means ± SD (n=6) and those of the same compound not sharing a common letter are significantly different (p<0.05).

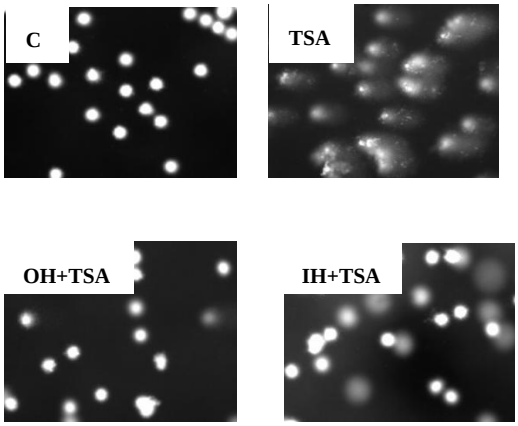
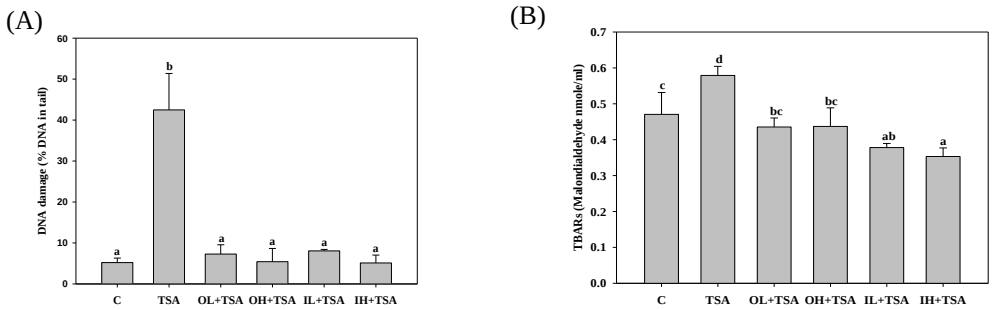


Figure 4. Lymphocyte DNA damage (A) and lipid peroxidation (TBARS level) (B) in tumor-bearing mice with various treatments. Values (means ± SD, n=6) not sharing a common letter are significantly different (p<0.05).



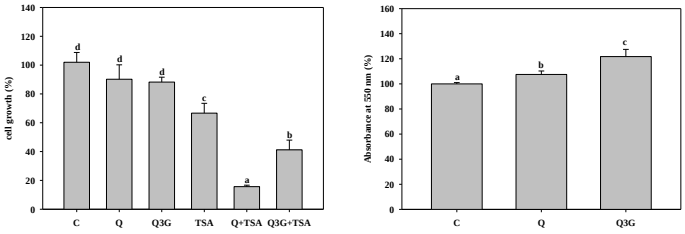


Figure 5. Effects quercetin (Q) or quercetin-3-glucuronide (Q3G) on the growth (A) or intracellular β-glucurgonidase activity (B) of A549 cells exposed to trichostatin A (TSA) or not. Values (means ± SD, n=3) not sharing a common letter are significantly different (p<0.05).

References

Chen W, Wang X, Zhuang J, Zhang L, Lin Y. Induction of death receptor 5 and suppression of survivin contribute to sensitization of TRAIL-induced cytotoxicity by quercetin in non-small cell lung cancer cells. Carcinogenesis. 28: 2114-2121, 2007.

Chan ST, Yang NC, Huang CS, Liao JW, Yeh SL. Quercetin enhances the antitumor activity of trichostatin A through upregulation of p53 protein expression in vitro and in vivo. PLoS One. 8:e54255, 2013.

Dokmanovic M, Marks PA. Prospects: histone deacetylase inhibitors. J Cell Biochem. 96:293-304, 2005.

Hertog MG, Hollman PC, Katan MB, Kromhout D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. Nutr Cancer. 20:21-29, 1993.

Jung YH, Heo J, Lee YJ, Kwon TK, Kim YH. Quercetin enhances TRAIL-induced apoptosis in prostate cancer cells via increased protein stability of death receptor 5. Life Sci. 86:351-357, 2010.

Kim HR, Kim EJ, Yang SH, Jeong ET, Park C, Lee JH, et al. Trichostatin A induces apoptosis in lung cancer cells via simultaneous activation of the death receptor-mediated and mitochondrial pathway. Exp Mol Med. 38:616-624, 2006.

Lee WJ, Chen YR, Tseng TH. Quercetin induces FasL-related apoptosis, in part, through promotion of histone H3 acetylation in human leukemia HL-60 cells. Oncol Rep. 25:583-591, 2011.

Sampson L, Rimm E, Hollman PC, de Vries JH, Katan MB. Flavonol and flavone intakes in US health professionals. J Am Diet Assoc. 102:1414-1420, 2002.

Seo SK, Jin HO, Woo SH, Kim YS, An S, Lee JH, et al. Histone deacetylase inhibitors sensitize human non-small cell lung cancercells to ionizing radiation

through acetyl p53-mediated c-myc down-regulation. J Thorac Oncol. 6: 1313-1319, 2011.

Shu-Ting Chan. Studies of quercetin on the antitumor and the side effects of trichostatin A. Doctoral Dissertation, 2015.

Shu-Ting Chan, Yi-Chin Lin, Cheng-Hung Chuang, Rong-Jen Shiau,Jiunn-Wang Liao, and Shu-Lan Yeh. Oral and Intraperitoneal Administration of Quercetin Decreased Lymphocyte DNA Damage and Plasma Lipid Peroxidation Induced by TSA In Vivo. *BioMed Research International (Journal of Biomedicine and Biotechnology)*, 2014:580626.

Zhang Y, Li Y, Cao C, Cao J, Chen W, Zhang Y, et al. Dietary flavonol and flavone intakes and their major food sources in Chinese adults. Nutr Cancer. 62:1120-1127, 2010.

Acknowledgement

科技部補助計畫衍生研發成果推廣資料表

日期:2015/10/14

科技部補助計畫	計畫名稱: 槲皮酮對組蛋白去乙醯酶抑制劑抗腫瘤功效的影響：體外及體內研究
	計畫主持人: 葉姝蘭
	計畫編號: 102-2320-B-040-011-MY2　　學門領域: 保健營養

無研發成果推廣資料

102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：102-2320-B-040-011-MY2
計畫名稱：槲皮酮對組蛋白去乙醯酶抑制劑抗腫瘤功效的影響：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	2	2	100%		獲邀在營養年會演講
		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	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	1	1	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	50%	篇	
		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	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	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%		
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		對植化素quercetin可以增強抗癌藥物的作用機轉及使用方式有確切結果					

字敘述填列。）	

	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	

科技部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）
   論文已發表一篇，尚有一篇整理中

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）
   本計畫確認quercetin增強去乙醯酶抑制劑抗腫瘤效果的重要機制，並確認不同補充方式的有效性，有相當大的學術價值，已在國內外研討會有發表，也在SCI期刊發表，預計還能再有一篇SCI期刊的發表，另外對臨床抗癌的實用性來說，亦應有相當大的助益。