行政院國家科學委員會專題研究計畫 成果報告

太平洋紫杉醇(Paclitaxel)誘導不同骨癌細胞之細胞凋亡機制探討及合併不同類黃酮造成癌症轉移相關蛋白的影響(第 3 年)

研究成果報告(完整版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 97-2314-B-040-002-MY3
執 行 期 間 ：99 年 08 月 01 日至 100 年 07 月 31 日
執 行 單 位 ：中山醫學大學醫學系

計 畫 主 持 人：呂克修
共 同 主 持 人：謝易修
計畫參與人員：博士班研究生-兼任助理人員：林巧雯

處 理 方 式 ：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中 華 民 國 100 年 10 月 29 日

Paclitaxel induces apoptosis in human osteosarcoma cell (HOS) through ERK1/2 and JNK pathway

Yih-Shou Hsieh[1], Shun-Fa Yang[2], Shu-Chen Chu[3], and Ko-Hsiu Lu[2,4,*]

[1]Institute of Biochemistry, [2]Institute of Medicine, Chung Shan Medical University, Taichung 402, Taiwan

[3]Department of Food Science, Central Taiwan University of Science and Technology, Taichung, Taiwan

[4]Department of Orthopaedic Surgery, Chung Shan Medical University Hospital, Chung Shan Medical University, Taichung 402, Taiwan

Abstract

Paclitaxel has been found to exhibit cytotoxic and antitumor activity. There is little information regarding the mechanisms of apoptotic-inducing effect of paclitaxel on human osteosarcoma HOS cells. We measured the effect of paclitaxel-induced apoptosis By DAPI stain on HOS cells. We also examined the pathway of paclitaxel on the apoptosis in HOS cells using Western blotting. We found that paclitaxel can induce dose dependent apoptosis of HOS cells. Furthermore, paclitaxel treatment resulted in Erk1/2 and JNK activation in HOS cells. Moreover, treatment of HOS cells with paclitaxel- induced a sustained activation of the phosphorylation of ERK1/2 and JNK, and inhibition of Erk 1/2 by U0126 (Erk1/2 inhibitor) significantly abolished the paclitaxel -induced apoptosis. These results suggest that paclitaxel-induced cell apoptosis via Erk 1/2 and JNK pathways in HOS cells.

Keywords: Paclitaxel, osteosarcoma, HOS, apoptosis, Erk 1/2, JNK, MAPK pathway

Introduction

Osteosarcoma is the most common primary malignant tumor of bone, especially in children. The principles of treatment of osteosarcoma have undergone dramatic changes in the past 20 years. Until recently, 5-year survival of 20% with surgical treatment alone was considered acceptable. This outcome suggested that 80% of the patients had pulmonary metastasis (perhaps undetectable) at the time of presentation. Hence, the chemotherapy is usually employed in the adjuvant situation to improve the prognosis and long-term survival. However, their values were limited by toxicity or lack of efficacy. Various inducers of apoptosis and the mechanisms of apoptosis have been reported. Many chemotherapeutic drugs induce cancer cell death through apoptotic pathways.

Paclitaxel (Taxol) was isolated from the bark of the Pacific yew *Taxus brevifolia* and was reported to have cytotoxic activity against a broad range of murine leukemia and solid tumors. Those solid tumors include chemotherapy-resistant epithelial ovarian cancer, advanced breast cancer, small cell and non-small cell lung cancer, and head and neck cancer. In vitro data, caspase-3 inhibitors reduced human osteoblastic Saos-2 cells from paclitaxel-induced apoptosis. Human osteogenic sarcoma U2OS cells increased caspase-3 activity after paclitaxel exposure. We had also demonstrated that paclitaxel inhibited N-acetyltransferase activity, DNA adduct

formation and gene expression in U2OS cells. Thus far, little is known regarding the mechanisms of apoptotic-inducing effect of paclitaxel on HOS cells. Although paclitaxel interacts with microtubules and induces apoptosis in various tumor cells, whether it can induce apoptosis in HOS cells is not very clear. Apoptosis signal transduction and execution require the action of the cascade of caspases. Human caspases- 1 to -10 have described that activation of caspase cascade is involved in chemicals- and agentsinduced apoptosis. Caspase-3 is an executioner caspase and relies on the action of the initiator caspases including caspase-8 and caspase-9 for its action. However, there is no available information to address the effects of paclitaxel on the cell cycle of HOS cells and caspase-3 on paclitaxel-induced apoptosis in human HOS cells. Furthermore, the mitogen-activated protein kinase (MAPK) family regulated the cellular responses to different extracellular stimuli and played as a multi-functional mediator of signal transduction processes, including cell death, differentiation, proliferation, and migration. Three major MAPK subfamilies have been define the extracellular regulated kinases 1/2 (ERK1/ 2), the p38, and the c-Jun N-terminal kinases 1/2 (JNK1/2). Each MAPK is activated through a specific phosphorylation cascade. The ERK1/2 cascade is associated with celldifferentiation, proliferation, and survival. Activation of ERK1/2 phosphorylation can induce cell cycle arrest and apoptosis, such as DNA damage. Therefore, the purpose of this study

was to investigate the effects and possible mechanism of paclitaxel on the cell cycle progression and regulatory molecules of HOS cells.

Materials and methods

Cells and paclitaxel treatment

Human osteosarcoma HOS cell line obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan), were cultured in minimum essential medium (MEM) (Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal calf serum, 1 mM glutamine, 1% penicillin/streptomycin 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids and 1 mM sodium pyruvate (Sigma, St. Louis MO, USA). The cell cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2. For paclitaxel treatment, appropriate amounts of stock solution of paclitaxel (Sigma, St. Louis MO, USA) were added into culture medium to achieve the indicated concentrations and then incubated with cells for indicated time periods, while DMSO solution without paclitaxel was used as blank reagent.

Microculture tetrazolium (MTT) assay

For cell viability experiment, a microculture tetrazolium (MTT) [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay

was performed to determine the cytotoxicity of paclitaxel. HOS cells were plated in 24-well plates at a density of 3×10^4 cells/well and were added to different concentrations of paclitaxel at 37°C for 24 h. After the exposure period, media were removed, followed by washing of the cells with phosphate-buffered saline (PBS). Then, the medium was changed and incubated with 20 mL MTT (0.5 mg/mL) for 4 h. The viable cell number/dish is directly proportional to the production of formazan, which following solubilization with isopropanol, can be measured spectrophotometrically at 563 nm.

DAPI Staining

Apoptosis was also quantitated by assessing nuclear changes using the nuclear binding dye DAPI (Sigma Chemical Co., St Louis, MO, USA). After treatment, cells were fixed and permeabilized with 2% (w/v) formaldehyde and 0.4% (w/v) Triton X-100, respectively. Staining was performed with 1μg/ml DAPI in PBS for 3 min, and chromatin fluorescence was observed under UV-light microscope.

Western blot analysis

PARP-1, Bid, Bcl-2, Bax, Caspase 3, caspase 8, caspase 9, Erk1/2, JNK and p38 protein expressions were measured by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot. In all analyses, protein concentration was standardized among samples. Briefly, aliquots of cell lysates containing 30 μg were separated by 10% SDS-PAGE. Electrophoresis was carried out at 20 V/cm for

120 min. The proteins in the SDS-polyacrylamie gel were transferred onto nitrocellulose. After washing the blotted nitrocellulose twice with water, membranes were blocked in freshly prepared PBS containing 5%.

Results

Figure 1

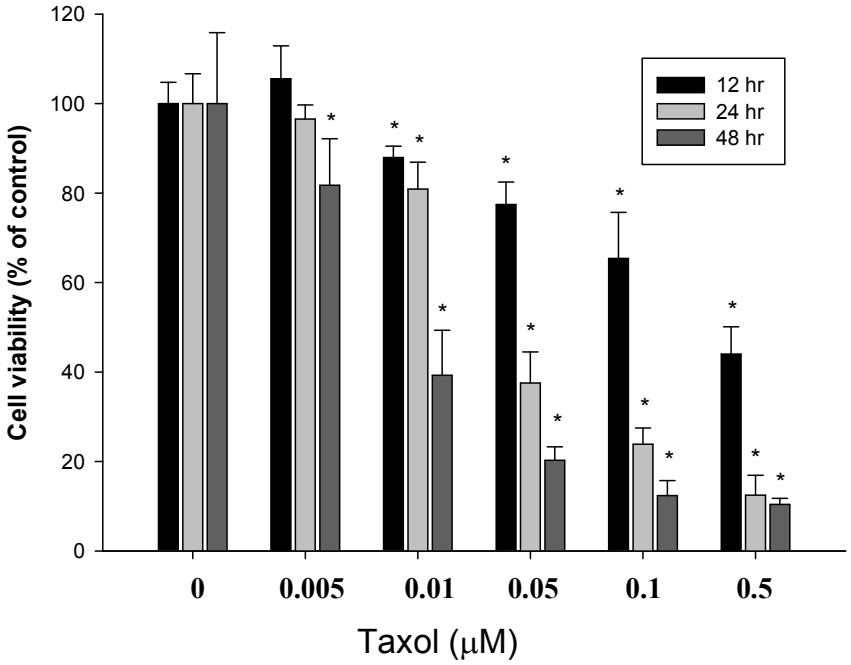

Figure 1. The effects of paclitaxel on viability of HOS cells. Cells were treated with paclitaxel at a concentration of 0.005, 0.01, 0.05, 0.1or 0.5 uM for 12, 24 and 48 hrs. At the end of treatment, cell viability was determined by MTT assay. The data are means ± SD of 3 independent experiments (*P < 0.05).

Figure 2

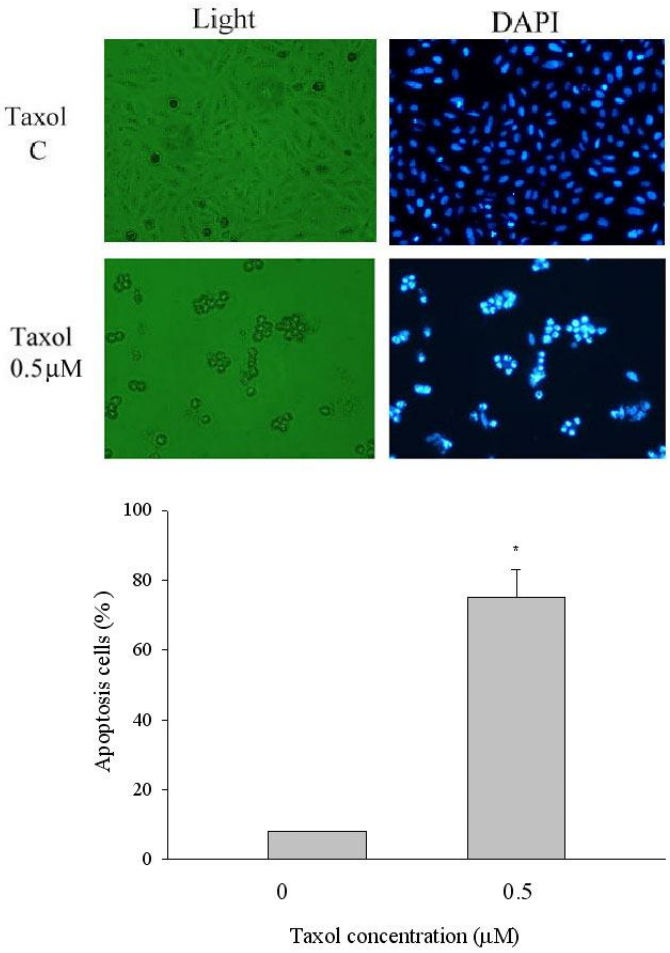

Figure 2. Apoptotic effect of paclitaxel on HOS cells. After a 48-hour paclitaxel treatment with 0.5 uM, DNA condensation was analyzed by DAPI staining. The data are means ± SD of 3 independent experiments (*P < 0.05)

Figure 3

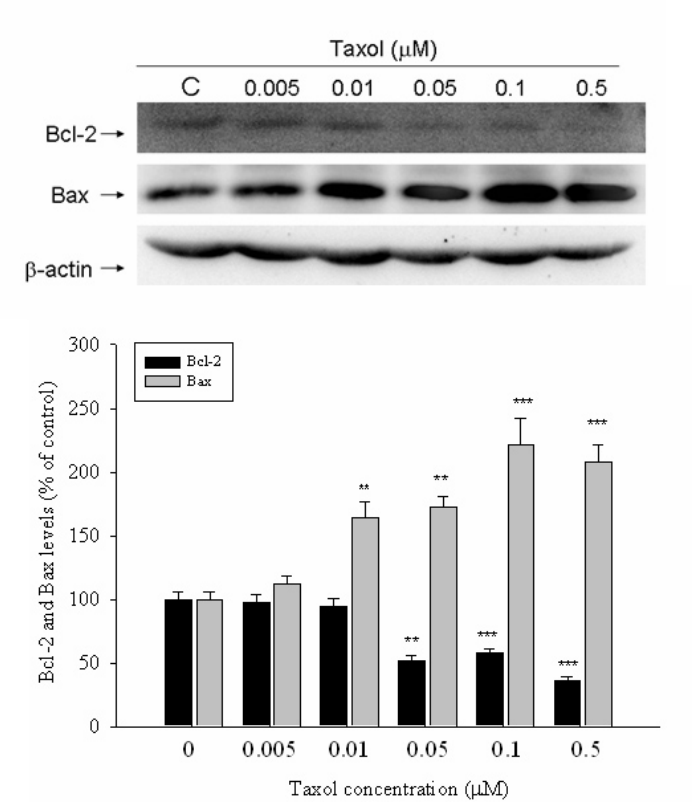

Figure 3. Effects of paclitaxel on the Bcl-2 and Bax of HOS cells. Cells were exposed to 0-0.5 uM paclitaxel for the indicated time, washed and then harvested. The cell lystaes were analyzed by western blotting. Paclitaxel induced the Bax expression and reduce Bcl-2 expression in HOS cells. The data are means ± SD of 3 independent experiments (*P < 0.05)

Figure 4

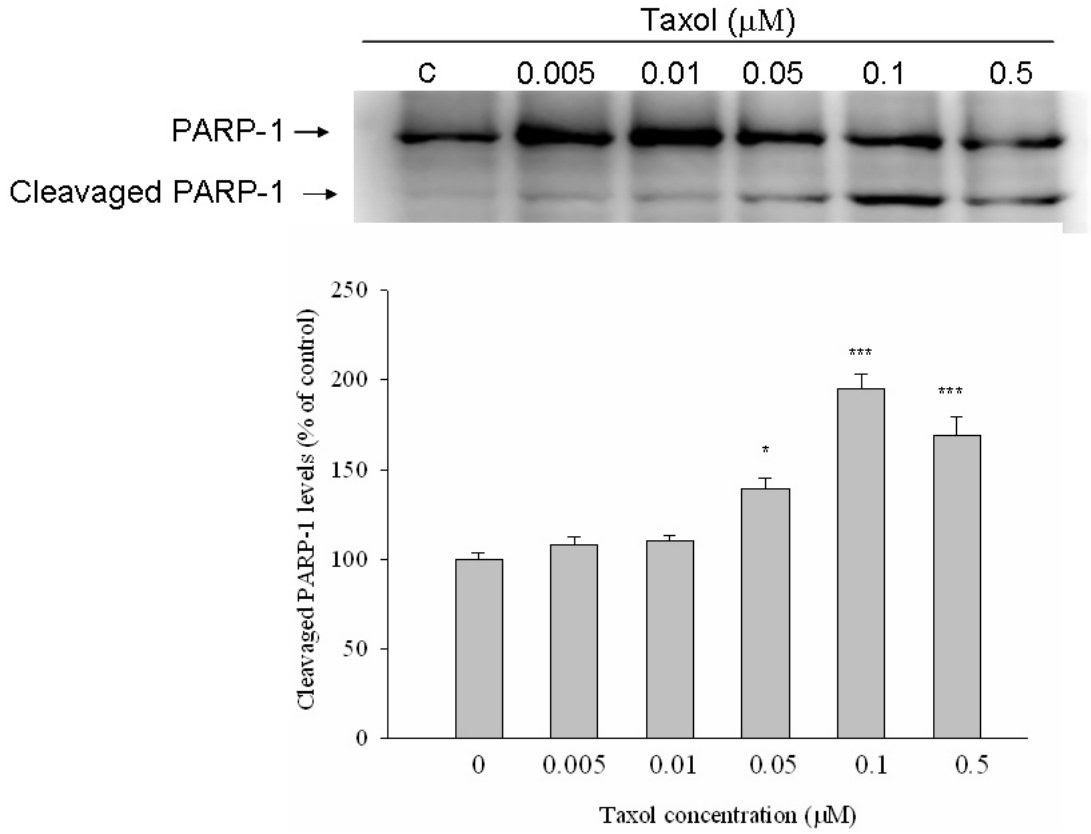

Figure 4. Effects of paclitaxel on the PARP-1 of HOS cells. Cells were exposed to 0-0.5 uM paclitaxel for the indicated time, washed and then harvested. The cell lystaes were analyzed by western blotting. Paclitaxel induced the PARP-1 cleavage in HOS cells. The data are means ± SD of 3 independent experiments (*P < 0.05)

Figure 5

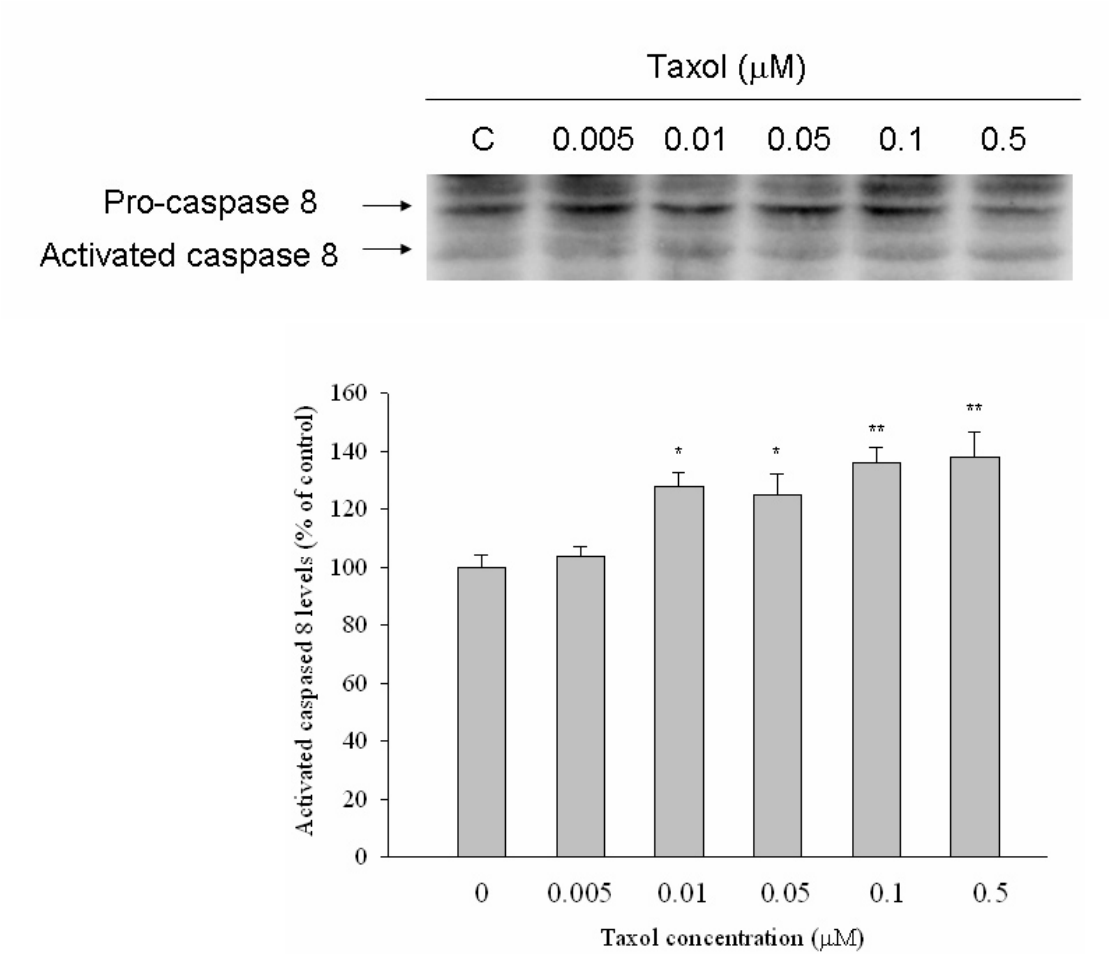

Figure 5. Effects of paclitaxel on the caspase 8 of HOS cells. Cells were exposed to 0-0.5 uM paclitaxel for the indicated time, washed and then harvested. The cell lystaes were analyzed by western blotting. Paclitaxel induced the caspase 8 activation in HOS cells. The data are means ± SD of 3 independent experiments (*P < 0.05)

Figure 6

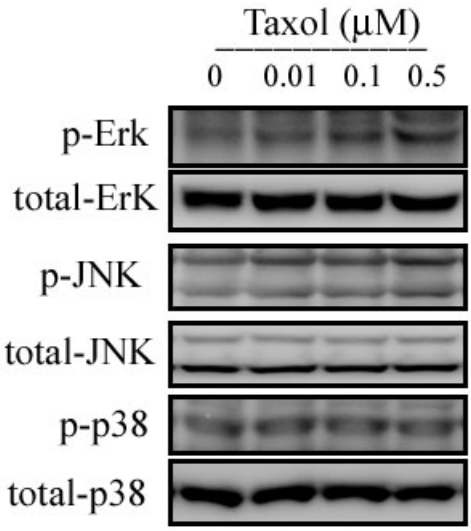

Figure 6. Effect of paclitaxel on MAPKs pathway. The effect of paclitaxel on the phosphorylation of ERK1/2, p38, and JNK1/2 was investigated by Western blot analysis. Paclitaxel induced ERK1/2 and JNK1/2 phosphorylation in HOS cells.

Figure7

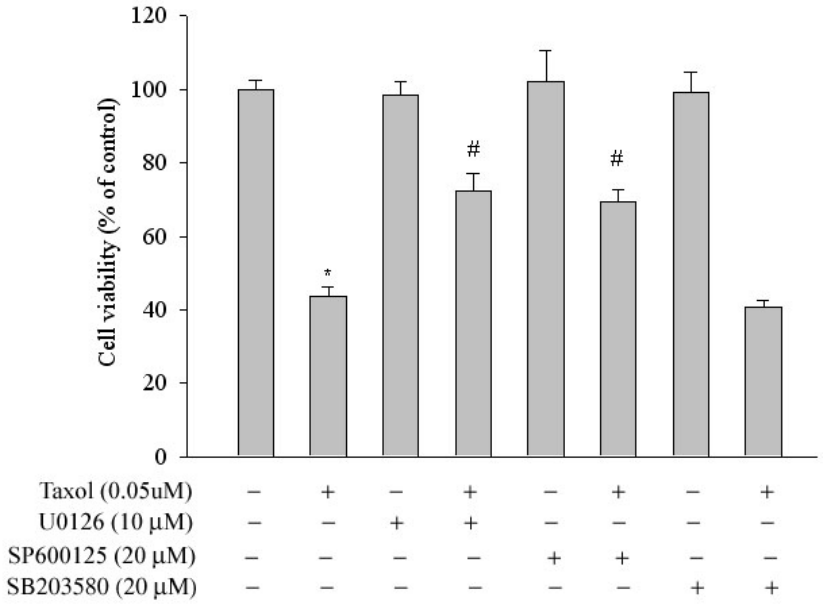

Figure 7. Effects of MAPK inhibitors on paclitaxel induced cell death. HOS cells were treated with 0.5 uM paclitaxel for 24 h in the presence or absence of 10 uM U0126, 20 uM SB203580, and 20 uM SP600125. Cell viability was estimated by MTT assay. The results were expressed as the percentage of cell viability. Data were represented as mean ± SD of three independent experiments performed in triplicate. *P<0.05, control versus paclitaxel; #P<0.05, paclitaxel versus MAPK inhibitor plus paclitaxel.

國科會補助計畫衍生研發成果推廣資料表

日期:2011/10/29

國科會補助計畫	計畫名稱: 太平洋紫杉醇(Paclitaxel)誘導不同骨癌細胞之細胞凋亡機制探討及合併不同類黃酮造成癌症轉移相關蛋白的影響
	計畫主持人: 呂克修
	計畫編號: 97-2314-B-040-002-MY3　　學門領域: 骨科

無研發成果推廣資料

97 年度專題研究計畫研究成果彙整表

計畫主持人：呂克修	計畫編號：97-2314-B-040-002-MY3
計畫名稱：太平洋紫杉醇(Paclitaxel)誘導不同骨癌細胞之細胞凋亡機制探討及合併不同類黃酮造成癌症轉移相關蛋白的影響	

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	0	0	100%		
		研討會論文	0	0	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	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%		
國外	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	3	3	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	1	1	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，請以文字敘述填列。)	無

	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)

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)

Paclitaxel 是一種從太平洋紫杉樹皮分離出來的成分,之前有許多文獻發現太平洋紫杉醇會誘導許多不同癌細胞的細胞凋亡。而我們之前的文獻也發現 Paclitaxel 會誘導 U2OS 這個人類骨癌細胞株走向凋亡，另外也會抑制細胞生長和細胞週期停止在 G2/M 期。但是對於另一株人類骨癌細胞株 HOS 的路徑仍不清楚。因此，我們利用 MTT assay 發現，Paclitaxel 會造成 HOS 的細胞死亡，而且有 dose and time-dependent 的效果。我們進一步利用 western blotting 發現，Paclitaxel 誘導 HOS 走向凋亡的路徑可能與 PARP-1, Bcl-2, Bax 及 caspase 8 這些傳統路徑有關，而 mitogen-activated protein kinase (MAPK) 此路徑也與細胞凋亡有關,因此,我們也發現 paclitaxel 造成人類骨癌細胞株 HOS 的凋亡與 Erk 1/2 及 JNK 等路徑有關。為了更進一步了解 paclitaxel 對此路徑的的影響，我們加入 Erk 1/2 及 JNK 的專一性抑制劑來確認其影響，結果證實了解 paclitaxel 對人類骨癌細胞株 HOS 的影響的確與 MAPK pathway 有關。因此，我們發現 MAPK 的活化的確在 paclitaxel 誘導人類骨癌細胞株 HOS 細胞凋亡過程扮演著重要的角色。未來在抗骨癌轉移的方向，MAPK pathway 也許是個可行的標的。