國立嘉義大學生命科學院
學生學術研究成果優良海報評選獲獎名單

時間：103 年 6 月 4 日

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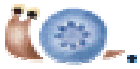

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Role of down-regulation of ERCC1 expression in metformin enhancing paclitaxel-induced cytotoxicity in human lung cancer cells

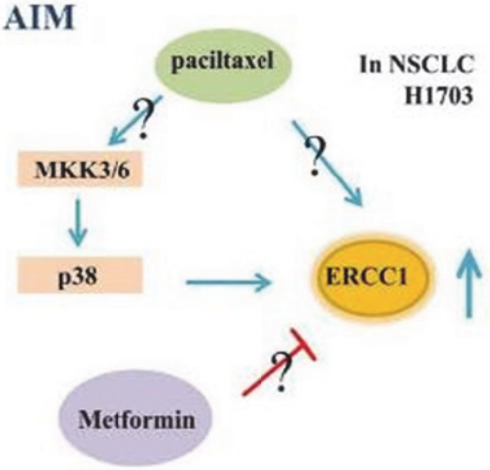
Yi-Jhen Huang, and Yun-Wei Lin*

Molecular Oncology Laboratory, Department of Biochemical Science and Technology, National Chiayi University, Chiayi, Taiwan

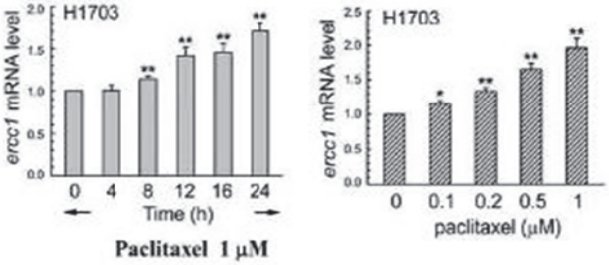
ABSTRACT

Metformin, a widespread used and well-tolerated biguanide class drug for treating type 2 diabetic patients, has recently discovered therapeutic effects on diversified human cancer cells. Paclitaxel (taxol), as a mitotic inhibitor isolated from the bark of the Pacific Yew tree and Taxus brevifolia, is an universal antitumor drug that can use in treatment of non-small cell lung cancer (NSCLC). Excision repair cross-complementary 1 (ERCC1) is a DNA excision repair protein. Whereas, overexpression levels of ERCC1 in cancer cells have been positively associated with the DNA repair capacity and repress cancer cells death to cause a poor prognosis in humans NSCLC treated with platinum-based chemotherapy. According to my preceding research, when paclitaxel used in human NSCLC cell lines H1703 cell, increased phosphorylation of mitogen-activated protein kinase (MAPK) kinase 3/6 (MKK3/6)-p38 MAPK as well as protein and mRNA levels of ERCC1. Moreover, down-regulation of p38 MAPK activation by either the pharmacological inhibitor SB202190 or p38 MAPK-siRNA was capable of enhancing NSCLC sensitivity to paclitaxel. In this research, we presumed that metformin raise the paclitaxel-induced cytotoxic effect by decreasing ERCC1 expression and MKK3/6-p38 MAPK activation. Together, our experiments demonstrated that down-regulation of ERCC1 expression and paclitaxel co-treated with metformin may provide varied lung cancer cells therapeutic effects in clinically useful combination.

AIM



RESULT



Paclitaxel 1 μM

Fig.1. Paclitaxel increased mRNA levels of ERCC1 in NSCLC.

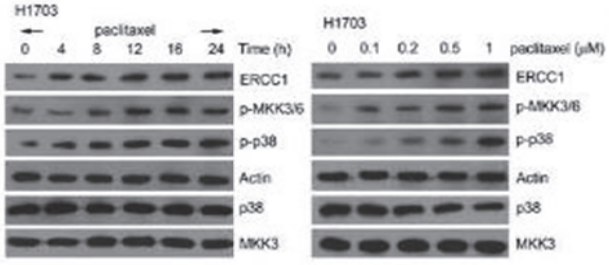


Fig.2. Paclitaxel increased protein levels of phospho-MKK3/6-p38 MAPK and ERCC1.

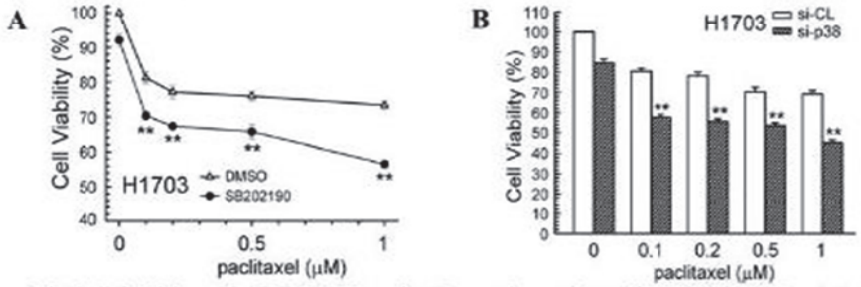


Fig.3. Inhibition of p38 MAPK activation enhanced paclitaxel-induced cytotoxicity.

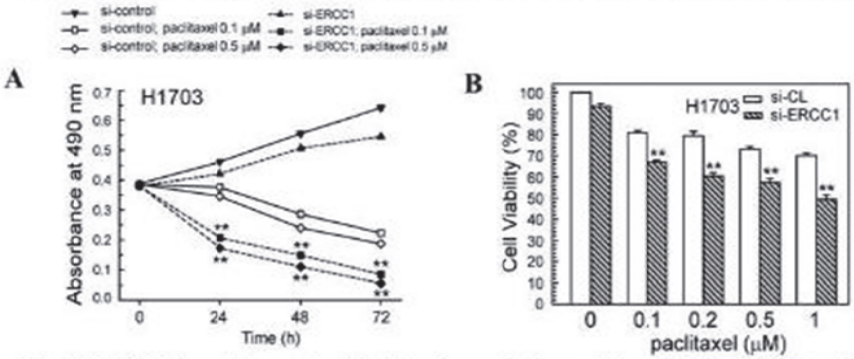


Fig.4. ERCC1 knockdown by siRNA enhanced the paclitaxel-induced cytotoxic effect.

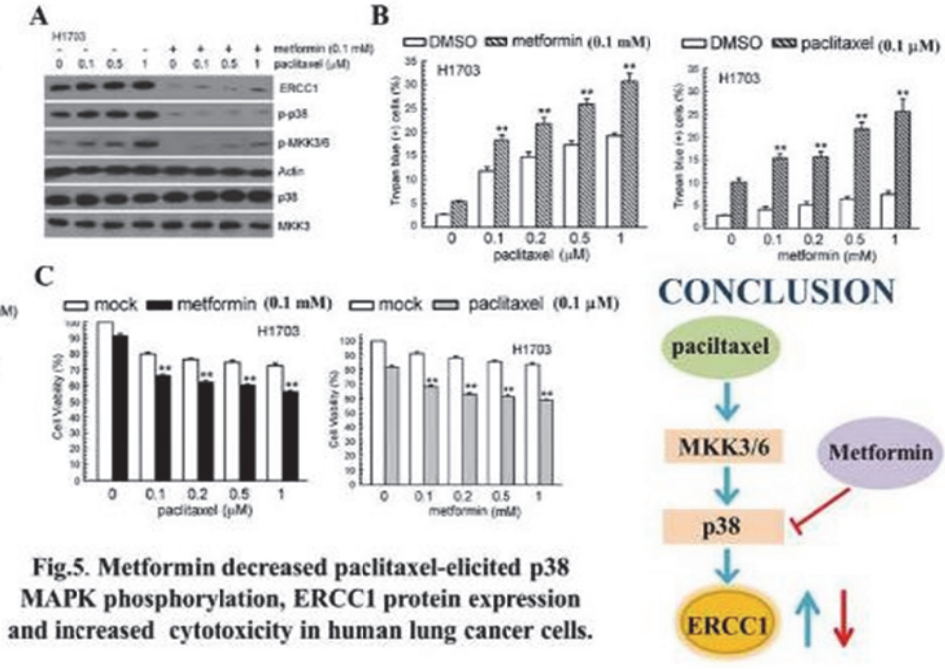


Fig.5. Metformin decreased paclitaxel-elicited p38 MAPK phosphorylation, ERCC1 protein expression and increased cytotoxicity in human lung cancer cells.

CONCLUSION

Regulatory effects of natural products on cell growth, melanin content and metastasis in melanoma cells

Yin-Chi Wu, Hui-Fen Liao*

Department of Biochemical Science and Technology, National Chiayi University

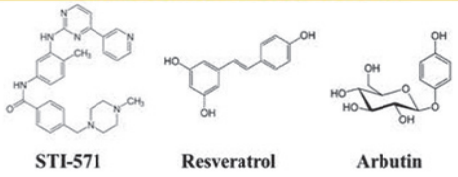
Introduction

Malignant melanoma can resistant to radiation therapy and chemotherapy, and has characteristics like high rate of metastasis and rapid cell growth. It makes the clinical therapy of malignant melanoma with the limited success. In previous experiments, we found that B16 melanoma cells with α-melanocyte-stimulating hormone (α-MSH) stimulation showed less sensitive to STI-571 (imatinib)-mediated cytotoxicity.

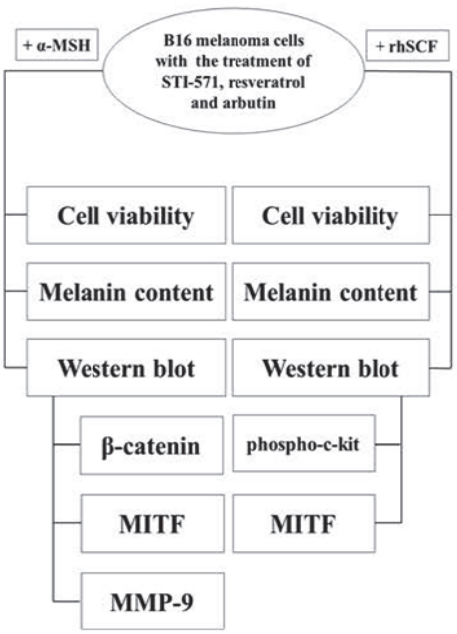
In melanoma cells, there is no definitive evidence that metastasis and drug resistance are correlation with melanin content. Therefore, the aim of this study assayed the correlation of natural products, such as resveratrol and arbutin, between melanin content and metastatic potency on B16 melanoma cells. We use α-MSH to promote melanin biosynthesis, for researching the effects of melanin biosynthesis on cell growth, drug resistance and metastasis in B16 melanoma cells, and analyzing the effects on melanin-related pathways, such as c-kit and Wnt/β-catenin pathways, as well as protein expression, including microphthalmia-associated transcription factor (MITF) and matrix metalloproteinases (MMPs).

We also use recombinant human stem cell factor (rhSCF), which can bind c-kit receptor on cell membrane, to activate c-kit signaling pathways, to further investigate the cell viability, melanin content, c-kit signaling pathway and MITF protein expression on B16 cells with the treatment of STI-571, resveratrol and arbutin.

Materials and Methods



STI-571 Resveratrol Arbutin



Results and Discussion

Cell viability assay:

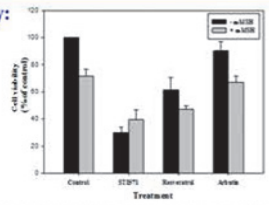


Fig 1. Effects of α-MSH-combined treatment with STI-571, resveratrol and arbutin on cell viability in B16 melanoma cells.

Melanin content:

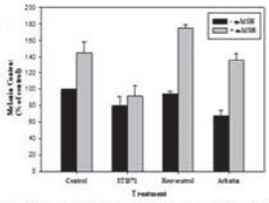


Fig 2. Effects of α-MSH-combined treatment with STI-571, resveratrol and arbutin on melanin content in B16 melanoma cells.

β-catenin expression:

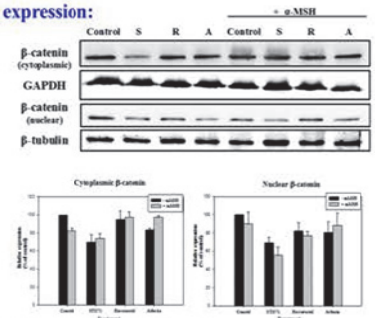


Fig 3. Expression of cytoplasmic and nuclear β-catenin in B16 melanoma cells by α-MSH-combined treatment with STI-571, resveratrol and arbutin.

MITF expression:

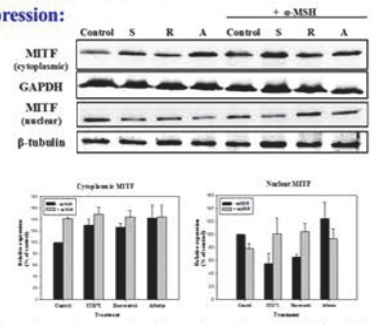


Fig 4. Expression of cytoplasmic and nuclear MITF in B16 melanoma cells by α-MSH-combined treatment with STI-571, resveratrol and arbutin.

MMP-9 expression:

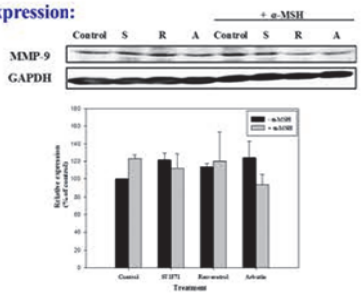


Fig 5. Expression of MMP-9 in B16 melanoma cells in α-MSH-combined treatment with STI-571, resveratrol and arbutin.

Stem cell factor (SCF) effect on viability of B16 cells:

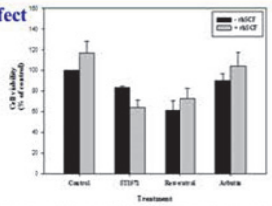


Fig 6. Effects of rhSCF-combined treatment with STI-571, resveratrol and arbutin on cell viability in B16 melanoma cells.

SCF effect on melanin content:

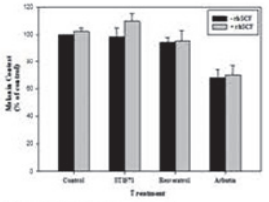


Fig 7. Effects of rhSCF-combined treatment with STI-571, resveratrol and arbutin on melanin content in B16 melanoma cells.

SCF effect on p-c-kit expression:

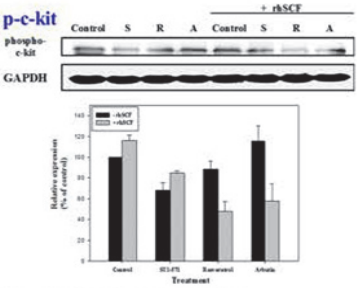


Fig 8. Expression of phospho-c-kit in B16 melanoma cells by rhSCF-combined treatment with STI-571, resveratrol and arbutin.

SCF effect on MITF expression:

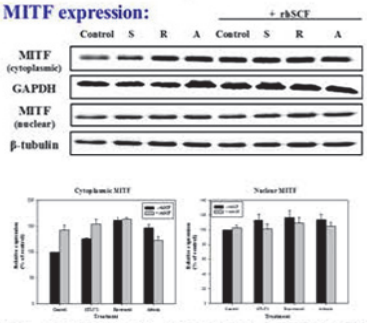


Fig 9. Expression of cytoplasmic and nuclear MITF in B16 melanoma cells by rhSCF-combined treatment with STI-571, resveratrol and arbutin.

Conclusions

- STI-571, resveratrol, and arbutin inhibited cell viability and melanin content, but didn't have significant effect on MMP-9 expression in B16 melanoma cells.
- STI-571 has inhibitory effects on nuclear β-catenin/MITF protein expression of B16 melanoma cells, suggesting that STI-571 may downregulate β-catenin-related pathways and inhibit downstream gene regulation, such as MITF.
- Resveratrol and arbutin have inhibitory effects on phospho-c-kit protein level in rhSCF-treated B16 melanoma cells, but have no significant effect on MITF protein level. It could be hypothesized that resveratrol and arbutin could downregulate rhSCF-activated c-kit signal pathways, but such effect may not regulate the expression of MITF.

探討菌桿孢芽粉澱化液對水稻紋病枯的防治潛力

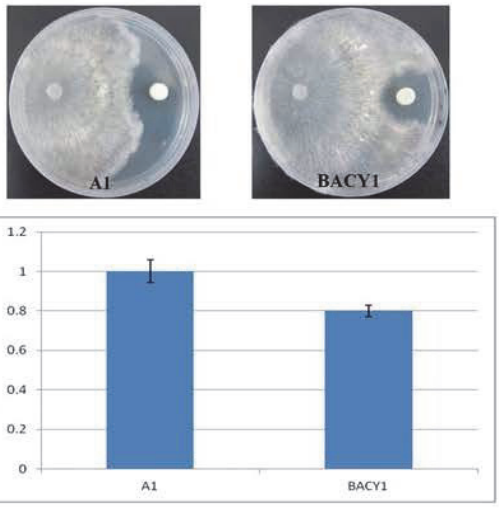
劉怡君、陳瑞祥

國立嘉義大學 生化科技學系

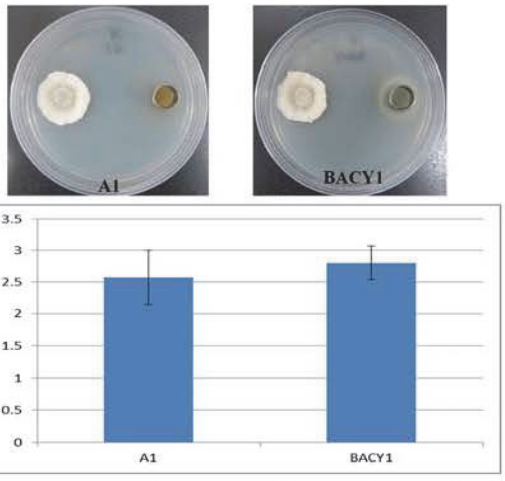
為，一之害病要主最稻水灣臺為病枯紋稻水探討菌桿孢芽粉澱化液稻水治防為作紋枯之劑製物生病潛力，本研究針對自臺灣本土分離所得之液化澱粉芽孢桿菌 *Bacillus amyloliquefaciens* BACY1 及 A1 菌株，測試其對水稻紋枯病菌的生長抑制作用。利用對峙培養測試其對紋枯病菌*Rhizoctonia solani*之菌絲生長抑制效果，結果發現使用500 ml脂胜肽萃取液皆有很明顯的抑制效果。另外利用掃描式電子顯微鏡觀察發現經過脂胜肽萃取液處理過的紋枯病菌，其菌絲或菌核皆呈現嚴重蜷曲萎縮狀，進而使得紋枯病菌無法正常生長。而液化澱粉芽孢桿菌是否能夠在田間施用對水稻紋枯病菌也有一樣的抑制效果，仍需進一步的研究試驗。

液化澱粉芽孢桿菌脂胜肽之萃取

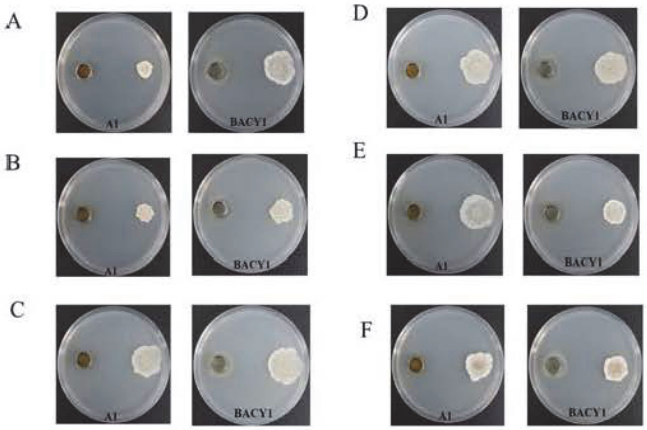
將液化澱粉芽孢桿菌保存管以接菌環沾取菌液於LB (Luria-Bertani Broth) 瓊脂培養基劃取單一菌落，於 28℃ 培養24小時。再挑出單一菌落至100 ml LB液態培養基，於150 rpm，28℃ 下培養至菌液濃度A_{600}達到0.4時，取500 ml菌液至Best培養基 (pH 7.0，1% tryptone 和 2.5% glucose) 於150 rpm，30℃ 震盪培養48小時，將培養菌液以12,000 rpm，4℃ 離心20分鐘，取出上清液以10 N HCl 至整調 pH 2.0液清上使，pH 於置，出析而點電等之肽胜脂於低值4℃ 下30以，後鐘分12,000 rpm，4℃ 心離20再，液清上除移，部底管心離至心離肽胜脂之出析將，鐘分以3 ml 50% 酒精以並，溶回 1 N NaOH至整調pH 7.0以後最，0.22 mm filter (Millex-GV Filter Unit, Millipore) 過濾。



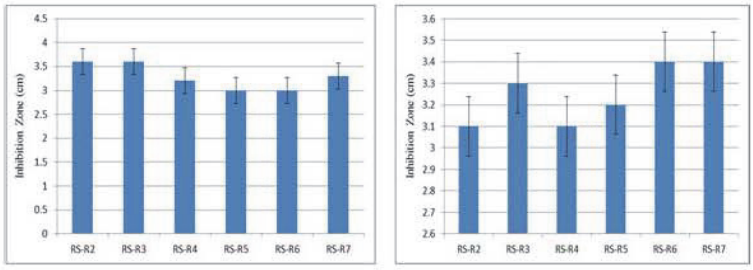
圖一、*Bacillus amyloliquefaciens* A1 及 BACY1 菌株脂胜肽 (處理量為 80 ml) 對紋枯病菌生長之抑制情形。



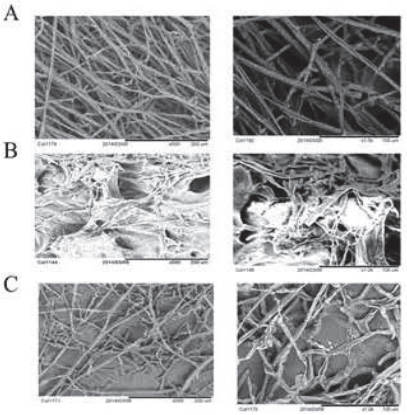
圖二、*Bacillus amyloliquefaciens* A1 及 BACY1 菌株脂胜肽 (處理量為 500 ml) 對紋枯病菌生長之抑制情形。



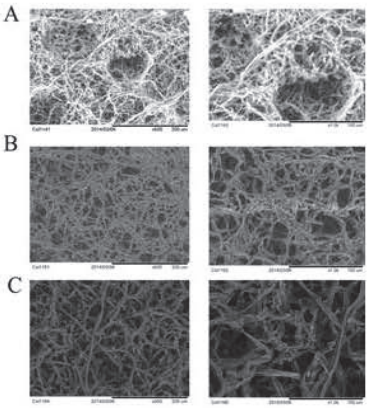
圖三、*Bacillus amyloliquefaciens* A1 及 BACY1 菌株脂胜肽 (處理量為 500 ml) 對不同紋枯病菌菌株生長之抑制情形 (A) 紋枯病菌RS-R2；(B) 紋枯病菌RS-R3；(C) 紋枯病菌RS-R4；(D) 紋枯病菌RS-R5；(E) 紋枯病菌RS-R6；(F) 紋枯病菌RS-R7。



圖四、*Bacillus amyloliquefaciens* (A) A1 及 (B) BACY1 菌株脂胜肽 (處理量為 500 ml) 對不同紋枯病菌菌株生長之抑制情形。



圖五、利用掃描式電子顯微鏡觀察液化澱粉芽孢桿菌脂胜肽對紋枯病菌絲之影響 (A) 未處理；(B) 脂胜肽萃取液處理；(C) 菌液處理。



圖六、利用掃描式電子顯微鏡觀察液化澱粉芽孢桿菌脂胜肽對紋枯病菌核之影響 (A) 未處理；(B) 脂胜肽萃取液處理；(C) 菌液處理。

結語

本研究結果顯示液化澱粉芽孢桿菌BACY1 及 A1 脂胜肽對紋枯病菌株皆有明顯的抑制效果，其抑制效果推論是藉由使菌絲嚴重蜷曲萎縮，破壞內部構造，進而使得無法正常生長。而液化澱粉芽孢桿菌是否能夠在土壤中對水稻紋枯病菌也有一樣的抑制效果，仍需進一步的研究試驗，若能成功有抑制效果，則可供日後開發水稻紋枯病菌病害防治用生物製劑的參考依據。

Study on removal of plant browning products and purification of a toxic protein with antineoplastic activity from *Bidens pilosa* var. *radiata*

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[1]Department of Biochemical Science and Technology & [2]Department of Microbiology, Immunology and Biopharmaceuticals ,National Chiayi University, 300 Syuefu Road, Chiayi County 60004, Taiwan

Abstract

Based on our previous study, extracts of *Bidens pilosa* var. *radiata* contain anti-proliferation activity to colorectal cancer cells. Due to a lot of browning products interfering subsequent experimental analysis and purification processes, it is necessary to overcame this issue by adding various chemicals to remove browning products. The cytotoxicity of different preparation of purified proteins from *Bidens pilosa* var. *radiate* is monitored with the SRB (Sulforhodamine B) assay. The protein extract was precipitated with 35%~65% saturated ammonium sulfate, and de-colored with polyvinyl- polypyrrolidone (PVPP), aluminum sulfate, polyethyleneimine (PEI), and polyethylene glycol (PEG) 4,000 plus ammonium sulfate aqueous two phase system, respectively. PEI shows the best de-colored effect on protein sample solution. The PEI is subsequent removed by a DEAE anion exchanger. The purified protein fraction still show high cytotoxicity. Therefore, dissolved ammonium sulfate precipitate solution is de-colored by PEI, and purified with DEAE Sepharose™ and Q Sepharose™ anion-exchanger for further characterization of toxin, named "bidenin".

Bidenin is separated and detected by the native or SDS polyacrylamide gel electrophoresis with the gel slicing technique. The estimated molecular weight of bidenin is identified by SDS-PAGE is about 88 kDa. The identification of bidenin will be completed by mass spectrometry in the coming future.

Method

圖片來源：彰化縣教育網路中心

DLD-1 + SRB assay = Cell viability

Cell viability (%) = (Sample OD_{560} / PBS OD_{560}) x 100%

Saturated Ammonium Sulfate Precipitate

↓

Remove of Browning Products

PVPP)	Aluminum sulfate	PEI	PEG 4,000

↓

Purification of Toxic Protein

Anion exchanger chromatography (DEAE & Q)	Electrophoresis (Native & SDS)

Conclusion

The result of remove for browning products , PEI show the best de-colored effect on protein sample solution and clearly analysis band on gel.

Toxic protein is successfully purified by DEAE Sepharose™ and Q Sepharose™ anion-exchanger then separated and located by Native and SDS PAGE with gel slicing technique remained its cytotoxicity, named "bidenin".The molecular weight of bidenin showed about 88 kDa on SDS-PAGE.

Results

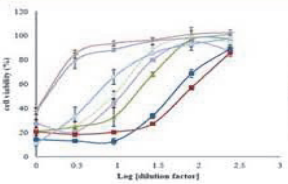

Fig.1. Analysis of the protein extracts precipitated with different range of ammonium sulfate on the cell viability of human colon adenocarcinoma cell (DLD-1).

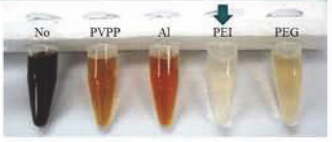

Fig.2. Effect of different agents on the color of ammonium sulfate precipitate dissolved protein solution.

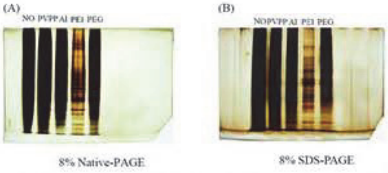

Fig.4. Native-PAGE (A) and SDS-PAGE (B) analysis of ammonium sulfate precipitate from each treating agents.

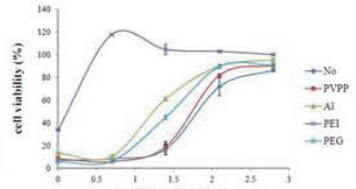

Fig.3. Use different agents as adsorbent to treating ammonium sulfate precipitate dissolved protein solution that effect on the cell viability of human colon adenocarcinoma cell (DLD-1).

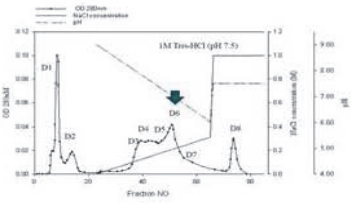

Fig.5.DEAE-Sepharose™ anion exchange chromatography of decolored protein solution.

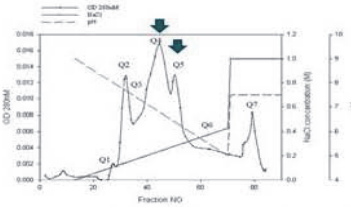

Fig.6.Q-Sepharose™ anion exchange chromatography of DEAE separated protein sample (D6).

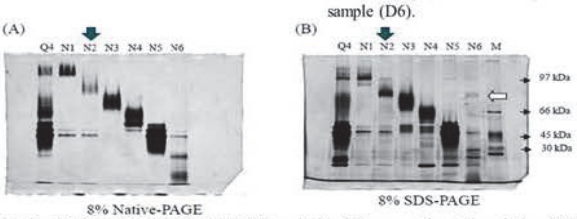

Fig.7. Native–PAGE (A) and SDS–PAGE (B) analysis of Q separated protein solution (Q4) purified by Native-PAGE extraction with gel slicing. Q4：Q separated protein sample, N1~N6：gel extracted protein solutions, M： L.M.W. protein marker.

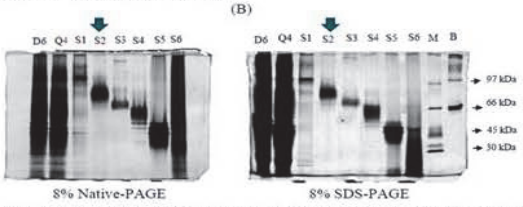

Fig.8. Native-PAGE (A) and SDS-PAGE (B) analysis Q separated protein solution (Q4) purified by SDS-PAGE extraction with gel slicing. D6：DEAE separated protein sample, Q4：Q separated protein sample, S1~S6：gel extracted sample solutions, M：L.M.W. protein marker, B：bovine serum albumin.

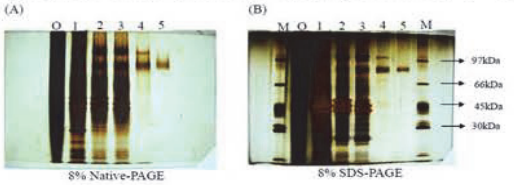

Fig.9. Native-PAGE and SDS-PAGE analysis of purification samples from each purification steps. O：ammonium sulfate precipitated protein solution； lane 1：PEI decolorized sample solution；lane 2：DEAE purified toxic sample；lane 3：Q purified toxic sample；lane 4：Native-PAGE gel slicing toxic protein sample (N2)；lane 5：SDS-PAGE gel slicing toxic protein sample (S2)；M：L.M.W. protein marker.

Role of p38 MAPK signal mediated MutS homologue-2 (MSH2) expression in regulating gefitinib and metformin-induced cytotoxicity in human lung squamous cancer cells

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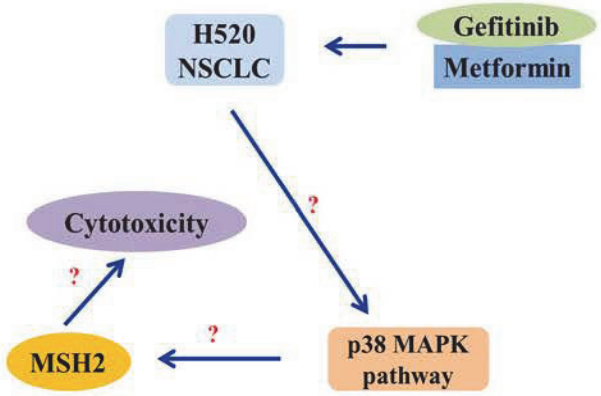
ABSTRACT

Gefitinib, a quinazoline-derived tyrosine kinase inhibitor, has anti-tumor activity *in vivo* and in *vitro*. Human MutS homologue-2 (MSH2) plays a central role in promoting genetic stability by correcting DNA replication errors. The present study investigated the effects of p38 mitogen-activated protein kinase (MAPK) signal on gefitinib-induced MSH2 expression in human non-small cell lung squamous cancer cell lines. Exposure of gefitinib increased MSH2 protein and mRNA levels, which was accompanied by MKK3/6-p38 MAPK activation in H520 cells. Moreover, knockdown of p38 expression by SB202190 and specific small interfering RNA (siRNA) significantly decreased gefitinib-induced MSH2 expression by increasing mRNA and protein instability. Enhancing p38 activation using constitutively active MKK6 (MKK6E) increased MSH2 protein and mRNA levels. Specific inhibition of MSH2 by siRNA significantly enhanced gefitinib-induced cytotoxicity. Metformin, an anti-diabetic drug, might reduce cancer risk. In human lung squamous cancer cells, metformin decreased gefitinib-induced p38 MAPK-mediated MSH2 expression and augmented the cytotoxic effect and growth inhibition by gefitinib. Transient expression of MKK6E or HA-p38 MAPK vector could abrogate metformin and gefitinib-induced synergistic cytotoxic effect in H520 cells. Together, metformin can down-regulate p38 MAPK-mediated MSH2 expression and enhance the cytotoxicity of gefitinib to human squamous lung cancer cells.

AIM



RESULT

Fig. 1. ***Gefitinib increased phospho-MKK3/6-p38 MAPK, MSH2 protein and mRNA levels in H520 cells.***

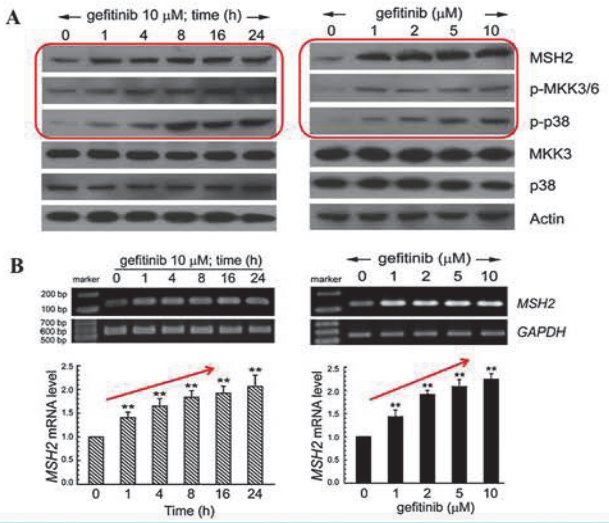


Fig. 2. ***p38 MAPK signaling pathway was involved in the gefitinib-induced increase in MSH2 protein and mRNA level.***

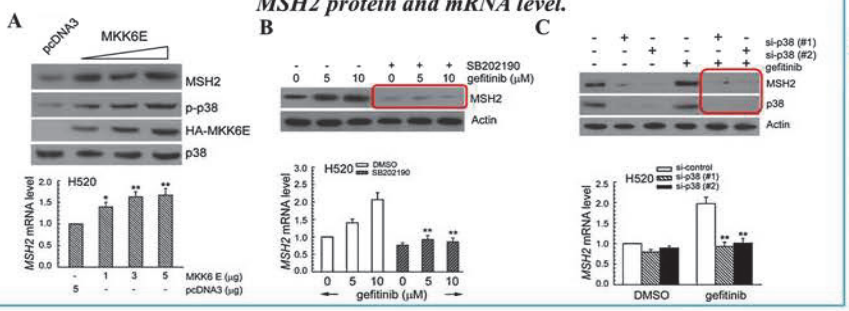


Fig. 3. ***Knockdown of MSH2 expression by si-RNA transfection or inhibition of p38 MAPK activation by SB202190 or specific si-p38 MAPK RNA transfection enhanced gefitinib-induced cytotoxicity.***

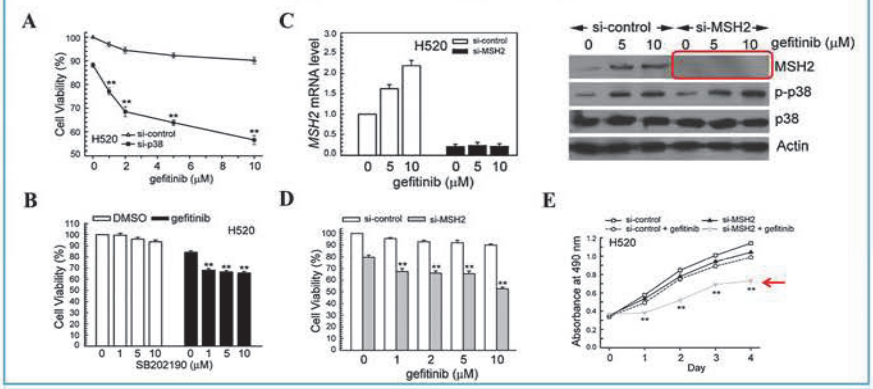


Fig. 4. ***Metformin decreased gefitinib-induced phospho-MKK3/6-p38 MAPK and MSH2 protein, and mRNA levels. and enhanced gefitinib-induced cytotoxicity.***

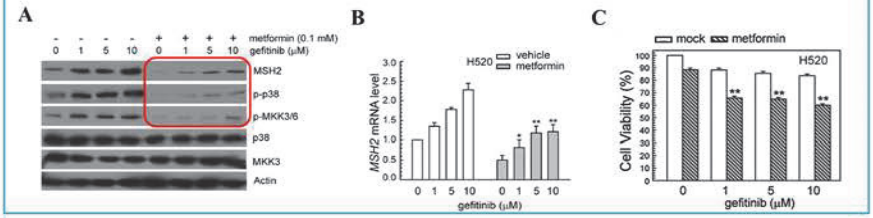
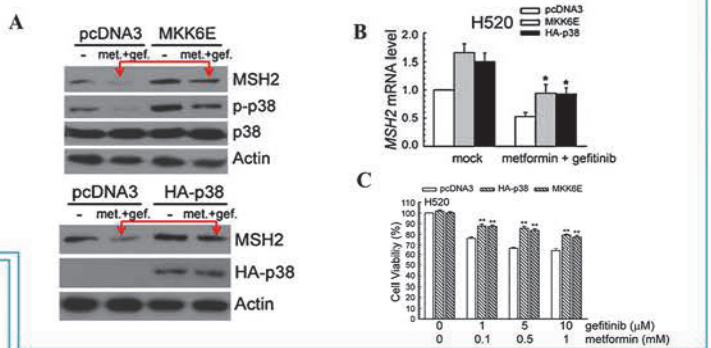
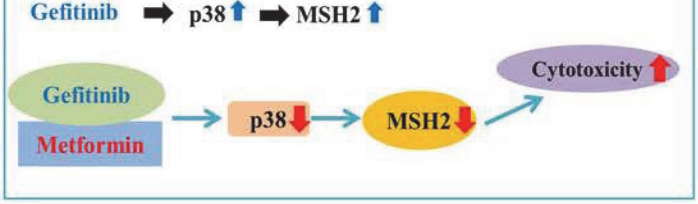


Fig. 5. ***Over-expression of MKK6E or HA-p38 MAPK restored the metformin-suppressed p38 MAPK activation and MSH2 protein expression in gefitinib-exposed H520 cells.***



CONCLUSION



Investigation of the molecular mechanism of tumorigenesis in 5-Fu- and Oxaliplatin-resistant human colorectal cancer stem cells

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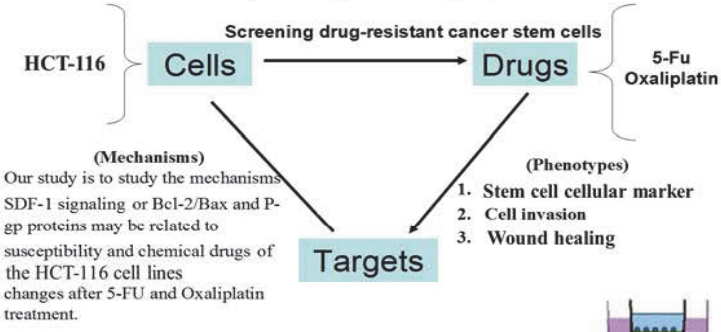
INTRODUCTION

Colorectal cancer in Taiwan is already the highest cancer incidence rate, and third cancer mortality rate. Chemotherapy treatments have made great contributions to the inhibition of cancer recurrence, and metastasis. But, Chemotherapy is often resistant in about half of the patients, resulting in cancer recurrence and progression. A lot of evidence are finding to suggest that cancer stem cells will lead chemoresistance and cause tumor sustained growth. However, colorectal cancer stem cells(CSCs) for drug resistance mechanisms remain unclear.

MOTIVE

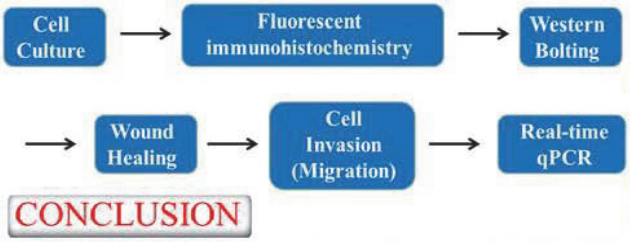
In this research, we use the mainstay chemotherapeutic drug, 5-Fluorouracil (5-FU) and Oaxliplatin (OxR) were selected with the colon cancer cell line HCT-116. The cancer stem cell lines were screened for colorectal cancer by the use of chemical resistant cloning method to analysis of whether a cancer stem cell characteristics. Study of the effect of SDF-1 on cancer stem cells signaling and uPA expression and the regulation of the Bcl-2/Bax and P-gp proteins may be related to resistant chemotherapy drug resistance mechanism.

Study Design



MATERIALS & METHODS

Colorectal cancer (CRC)	Cancer stem cell (CSC)
HCT-116	HCT-116/OxR HCT-116/5FU



CONCLUSION

This result finds that chemoresistant cells were enriched for CSC markers and showed higher tumorigenic capacity in vitro. Next, we will be determined that effects of 5-FU-resistant and OxR-resistant colorectal cancer stem cells were associated with overexpressed Bcl-2/Bax, P-gp and uPA proteins via activation of CXCL12 (SDF-1)/ CXC chemokine receptor 4 (CXCR4). These findings provide further insight into the molecular mechanism and suggest promising biomarkers for clinic therapy of colorectal cancer patients outcome of patients with lymph node metastasis.

REFERENCE

1. Anthony D. Yang, Fan Fan, E. Ramsay Camp, et al. (2006) Chronic Oxaliplatin Resistance Induces Epithelial-to-Mesenchymal Transition in Colorectal Cancer Cell Lines. *Clin Cancer Res.* 12:4147-4153.
2. You-Kyung Jeon, et al. (2010) Promoter hypermethylation and loss of CD133 gene expression in colorectal cancers. *World J Gastroenterol.* 16(25): 3153-3160.
3. Sanchita Roy, Adhip P N Majumdar.(2012). Signaling in colon cancer stem cells. *Journal of Molecular Signaling.* 7:11.

RESULTS

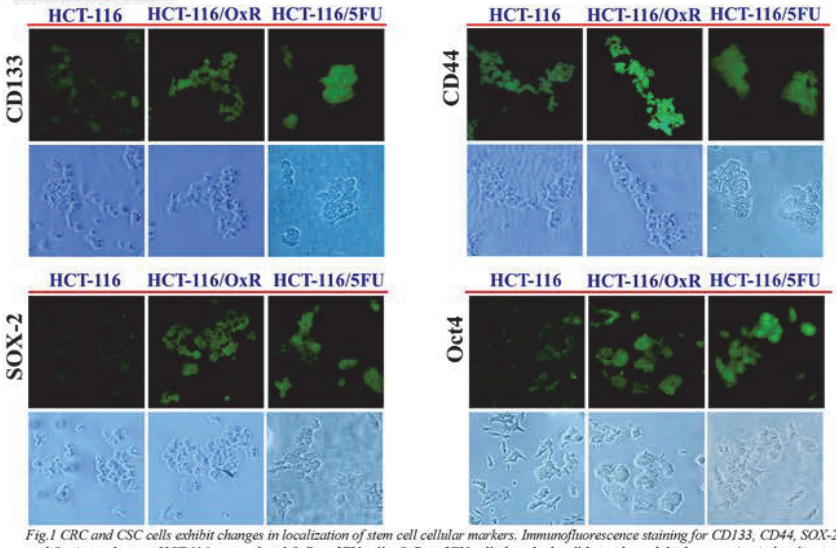


Fig.1 CRC and CSC cells exhibit changes in localization of stem cell cellular markers. Immunofluorescence staining for CD133, CD44, SOX-2 and Oct4 was done on HCT-116 parental and OxR or 5FU cells. OxR or 5FU cells from both cell lines showed high expression in localization of cellular markers.

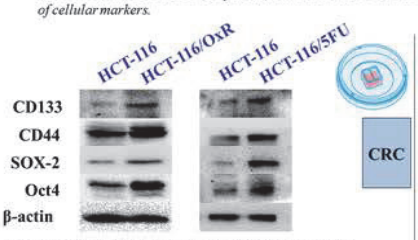


Fig.2 CRC and CSC cells of stem cell cellular markers by Western blotting analysis.

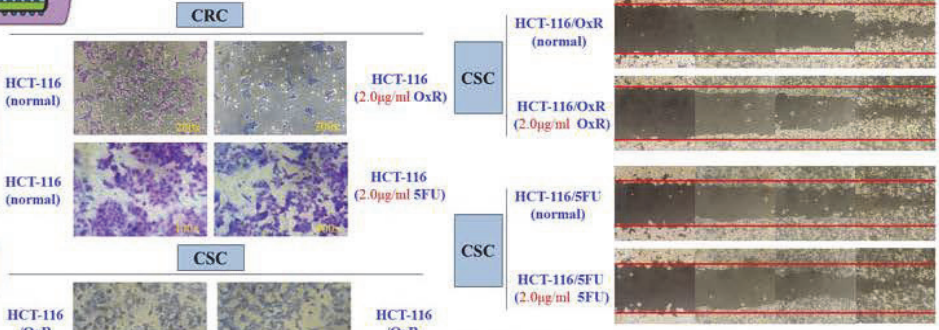


Fig.3 Wound-healing assay showed that the comparison of CRC and CSC cells.

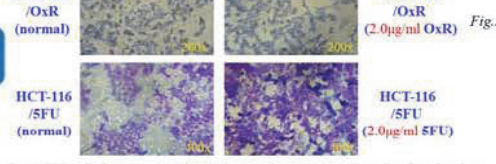


Fig.4 CSC cells have increased migratory and invasive capacity. Boyden chamber and modified Boyden chamber assays were done to compare the migratory and invasive capabilities of CRC and CSC cells.

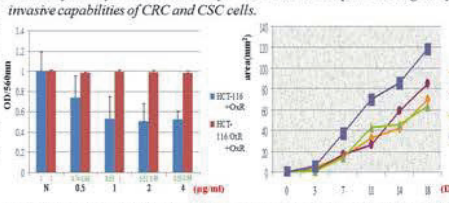


Fig.5 Compared drug resistance differentially expressed of the CRC and CSC cells in vitro/ in vivo.

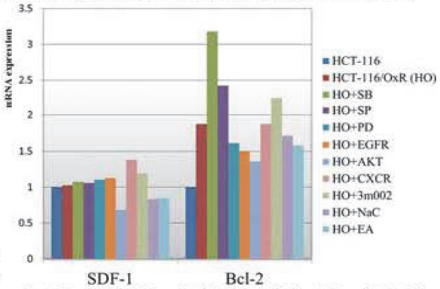


Fig.6 Compared SDF-1 and Bcl-2 expressed of the CRC and CSC cells in treatment of all kind inhibitors.