國立嘉義大學生命科學院 105 年度
學生學術研究成果優良海報評選獲獎名單

時間：105 年 6 月 1 日

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生化科技學系

Upregulation of amphiregulin expression promotes malignant progression in chondrosarcoma cells

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Abstract

Chondrosarcoma is a malignant type of bone cancer that is characterized by the production of cartilage matrix. The most lethal aspect of chondrosarcoma arises from the metastatic dissemination of primary tumors. Clinically, surgical resection is still the primary mode of treatment for chondrosarcomas. However, High-grade chondrosarcoma is not only cause of death due to resistance to conventional chemotherapy but also like to metastasize to other area of the body. Currently, there are no targeted drug therapies available for patients with metastatic chondrosarcomas. Doxorubicin (Dox) is one of the most widely used antitumor drugs, and has been shown to improve survival significantly compared with no treatment in chondrosarcoma.

At first, we established a migration-prone cell from JJ012 cells (designated JJ012-M). By comparing the secretomes of JJ012 and JJ012-M cells using two-dimensional gel electrophoresis, we found that amphiregulin (AR), a ligand of the epidermal growth factor receptor (EGFR), was highly secreted by JJ012-M cells. Knockdown of AR by lentiviral-mediated RNAi markedly suppressed cell migration and also increased Dox sensitivity in JJ012-M cells. In addition, we also established a Dox-resistant SW1353 subline (designated SW1353R). AR expression was significantly increased in SW1353R cells. Silencing of AR in SW1353R cells restored Dox sensitivity and also inhibited cell motility. Upregulation of AR might promote malignant progression in chondrosarcoma cells through epithelial-mesenchymal transition. To explore AR-induced signaling, we conducted a phospho-kinase array to determine which pathways are regulated by AR. Furthermore, the results of tissue array indicate that elevated AR expression is positively correlated with the grade of chondrosarcoma. Taken together, our data suggest that targeted inhibition of AR-regulated pathways may be an effective therapeutic strategy for controlling metastasis and Dox resistance in chondrosarcoma.

Results

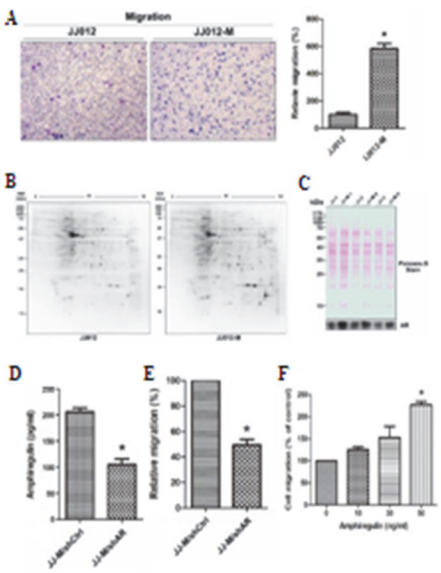


Figure 1. The expression level of AR is involved in cell migration in JJ012 cells.

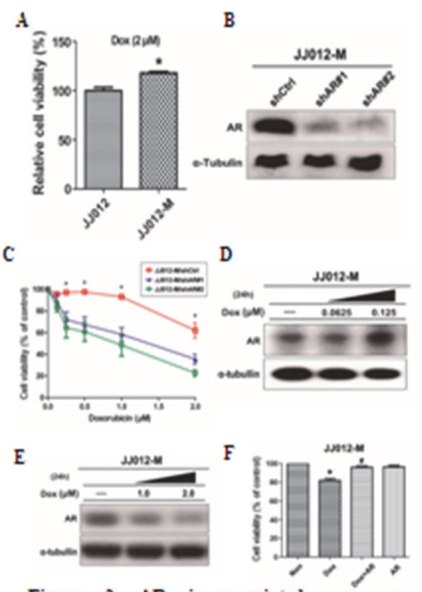


Figure 2. AR is associated with Dox resistance in JJ012-M cells.

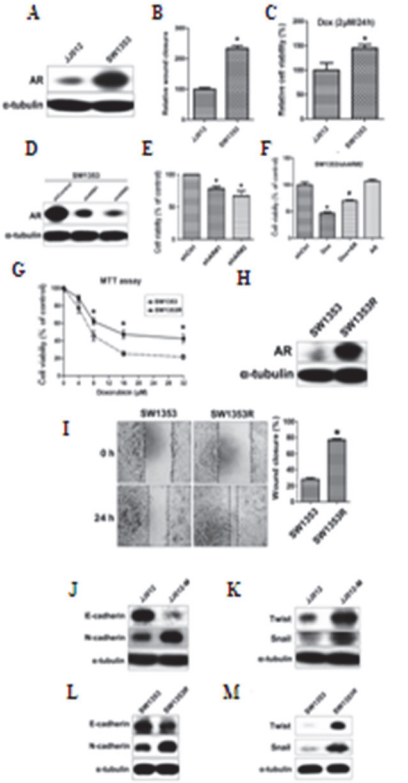


Figure 3. AR modulates cell migration and Dox sensitivity via EMT in JJ012 and SW1353 cells.

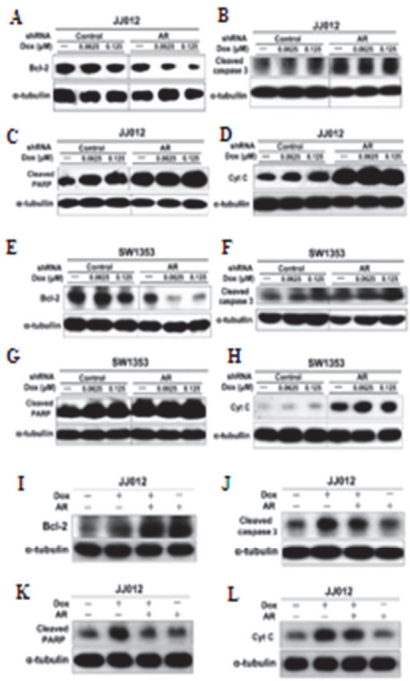


Figure 4. AR attenuates Dox-induced apoptosis in JJ012 and SW1353 cells.

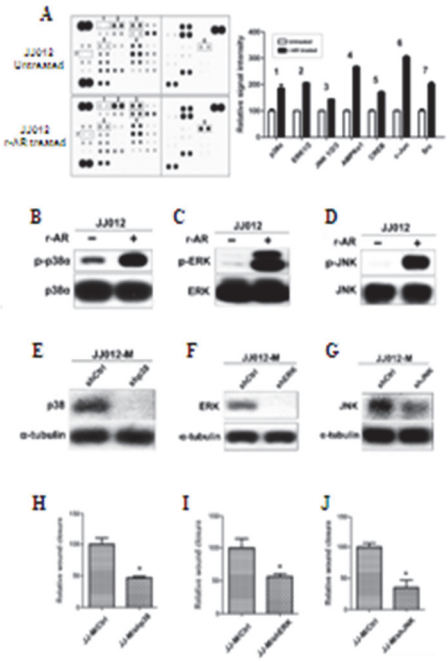


Figure 5. Phosphoproteomic analysis of recombinant AR (r-AR)-treated JJ012 cells.

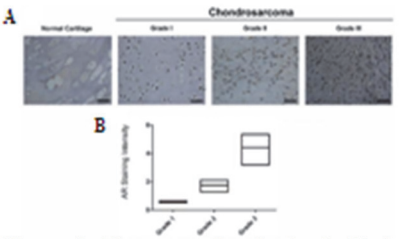
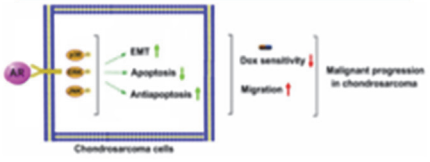


Figure 6. The expression level of AR is positively correlated with histopathological grade in human chondrosarcoma tissues.

Summary



Molecular mechanism down-regulation x-ray repair cross-complement group1 protein (XRCC1) expression by Hsp90 inhibition enhances the gefitinib-induced cytotoxicity in human lung cancer cells

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ABSTRACT

A selective epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), gefitinib (IressaR, ZD1839), blocks growth factor-mediated cell proliferation and extracellular signal-regulated kinase1/2 (ERK1/2) and AKT signaling activation. It has been shown that inhibition of Hsp90 function can enhance antitumor activity of EGFR –TKI. XRCC1 is an important scaffold protein in base excision repair, which could be regulated by ERK1/2 and AKT pathway. However, the ERK1/2 induced cytotoxicity in non-small cell lung cancer (NSCLC) has not been identified. In this study, gefitinib treatment decreased XRCC1 mRNA expression through ERK1/2 and AKT activation in A549 cells. Knocking down XRCC1 expression by transfection with small interfering RNA of XRCC1 enhanced the cytotoxicity and cell growth inhibition of gefitinib. Combining treatment of gefitinib with an Hsp90 inhibitor resulted in enhancing the reduction of XRCC1 protein and mRNA levels in gefitinib-exposed A549 cells. Compared to a single agent alone, gefitinib combined with an Hsp90 inhibitor resulted in cytotoxicity and cell growth inhibition synergistically in NSCLC cells. Furthermore, transfection with constitutive active MKK1 or AKT vectors rescued the XRCC1 protein level as well as the cell survival suppressed by an Hsp90 inhibitor and gefitinib. These finding suggested that down-regulation of XRCC1 can enhance the sensitivity of gefitinib for NSCLC cells.

Results

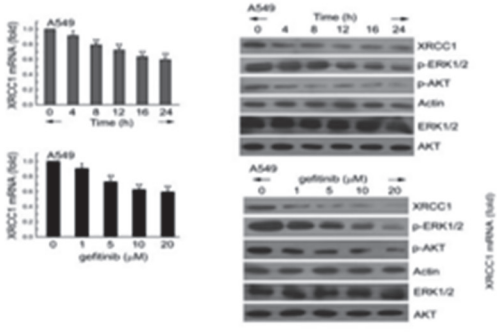


Fig. 1 - Gefitinib decreased XRCC1 expression in a dose and time-dependent manner.

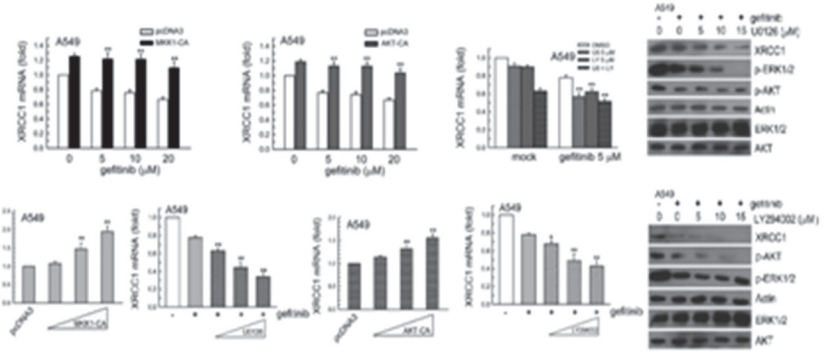


Fig. 2 - Gefitinib decreased XRCC1 expression via ERK1/2 and AKT inactivation in NSCLC cells.

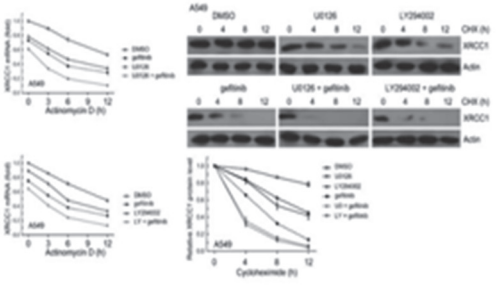


Fig. 3 - Gefitinib decreased XRCC1 mRNAand protein stability in NSCLC cells.

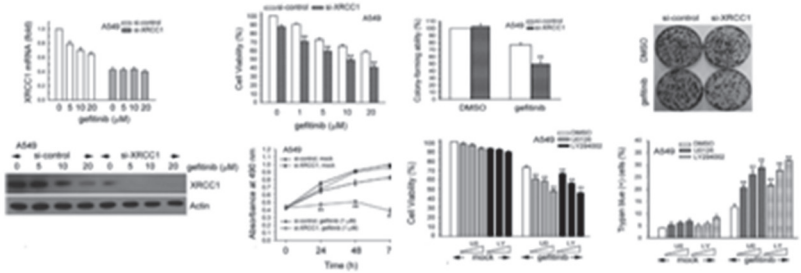


Fig. 4 – Knockdown of XRCC1 expression by si-RNA transfection enhanced the cytotoxicity induced by gefitinib.

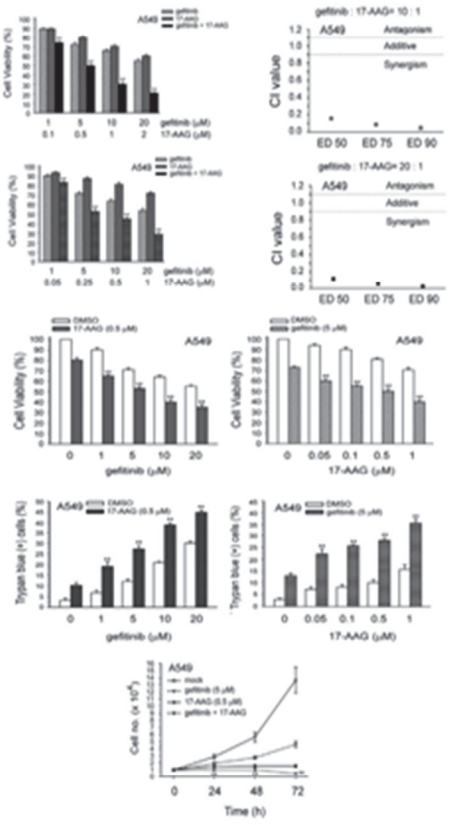


Fig. 5 - 17-AAG co-treatment with gefitinib synergisticallyenhanced cytotoxicity.

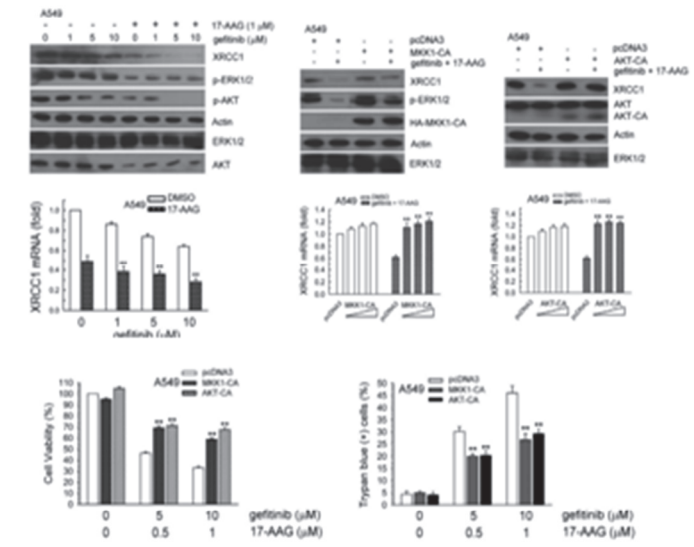
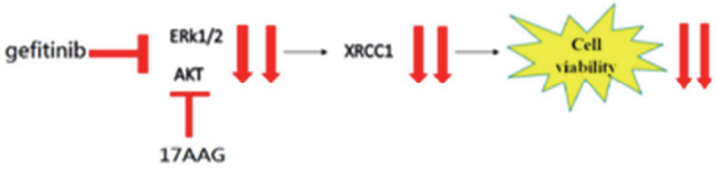


Fig. 6 – 17-AAG further decreased XRCC1 protein and mRNAlevels in gefitinib-exposed NSCLC cells.

Summary



Baicalein-loaded liposomes may inhibit ADM-induced steatosis in a cell model of human dermal fibroblasts

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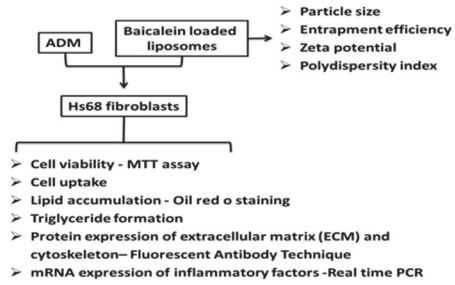
Introduction

Steatosis is the process describing the abnormal retention of lipids within a cell. Steatosis may occur in any organ and has been reported to raise some risky health problems. Hepatic steatosis may cause non-alcoholic steatohepatitis, which can progress to cirrhosis and hepatocellular carcinoma. Cardiac steatosis has been considered to increase the risk of heart disease. However, dermal steatosis has received much less attention and hence the responsible mechanisms for dermal steatosis still remain unclear. In this study, Hs68 fibroblasts were used as a cell model to investigate dermal steatosis.

Baicalein, a type of flavonoids, is extracted from the root of *Scutellaria baicalensis*. Baicalein appears to have a variety of bio-activities, including high anti-oxidant, anti-inflammatory, anti-proliferative, anti-apoptotic and anti-tumor activities. Previous studies have also proved that baicalein exhibited the inhibitory effect on lipid accumulation and adipocyte differentiation by suppressing early adipogenic factors and regulating m-TOR signaling pathway.

Therefore, we encapsulated baicalein in liposomes to measure the effect on adipogenic differentiation medium (ADM)-induced dermal steatosis in the cell model of Hs68 fibroblasts.

Methods



Results

Table 1. Physical properties of liposomal formulations

Drug formulation	Particle size (nm)	Entrapment (%)	PDI	Zeta potential (mV)
Empty liposomes	171.67±8.92		0.546	-1.69
Baicalein liposomes (10 μ/ml)	154.17±2.34	33.65%	0.503	-2.11
Baicalein liposomes (20 μ/ml)	135.67±3.45	25.40%	0.462	-1.89

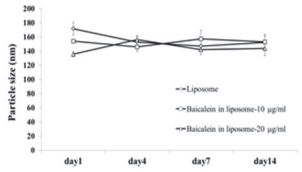


Figure 1. The stability of empty and baicalein-loaded liposomes.

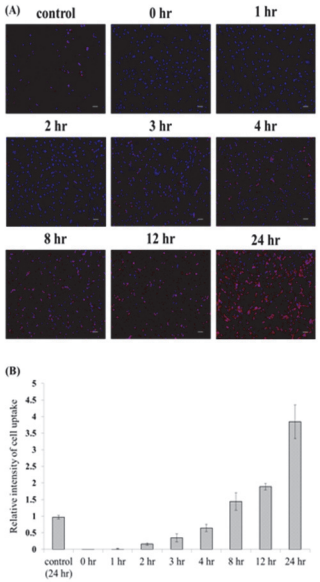


Figure 2. Cellular uptake of DiI-loaded liposomes in Hs68 fibroblasts. (A) Hs68 fibroblasts were incubated with DiI-loaded lipsomes at different time intervals (0-24 hours) (B) Quantification for cellular uptake of DiI-loaded liposomes. The results were presented in the ratio index in comparison with control.

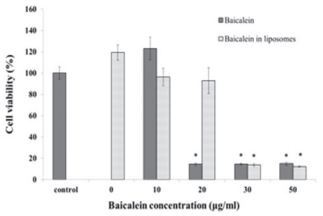


Figure 3. The cytotoxic effect of baicalein and baicalein-loaded liposomes on Hs68 fibroblasts. * p<0.05 with respect to control.

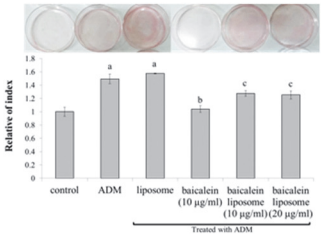


Figure 4. The effect of baicalein-loaded lipsomes on lipid accumulation of ADM-induced Hs68 fibroblasts after 14 days of incubation. a: p<0.05 relative to control, b: p<0.05 relative to cells treated with ADM, c: p<0.05 relative to cells treated with liposomes.

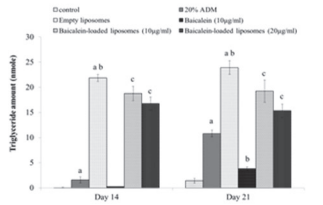


Figure 5. Baicalein and baicalein-loaded liposomes inhibited triglyceride generation after 14 and 21 days of ADM treatment. a: p<0.05 relative to control, b: p<0.05 relative to cells treated with ADM, c: p<0.05 relative to cells treated with liposomes.

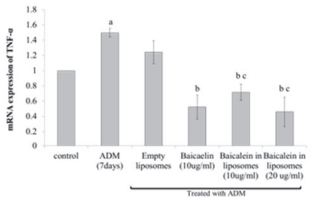


Figure 6. The inhibitory effect of baicalein-loaded liposomes on ADM-induced mRNA expression of TNF-α. a: p<0.05 relative to control, b: p<0.05 relative to cells treated with ADM, c: p<0.05 relative to cells treated with liposomes.

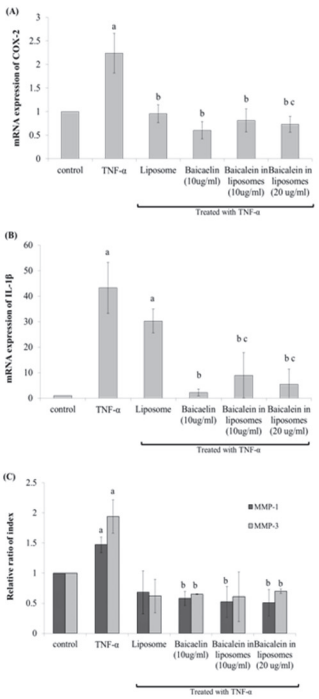
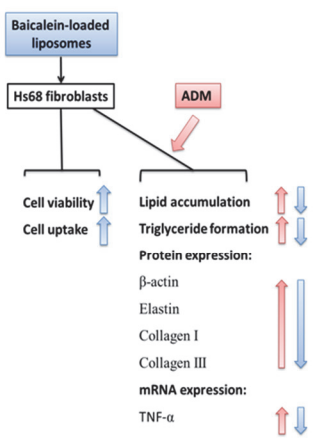


Figure 7. The effect of baicalein-loaded liposomes on gene expressions of inflammatory cytokines in TNF-α induced Hs68 fibroblasts. (A) Gene expression of COX-2. (B) Gene expression of IL-1β. (C) Gene expression MMP-1 and MMP-3. a: p<0.05 relative to control, b: p<0.05 relative to cells treated with ADM, c: p<0.05 relative to cells treated with liposomes.

Conclusion



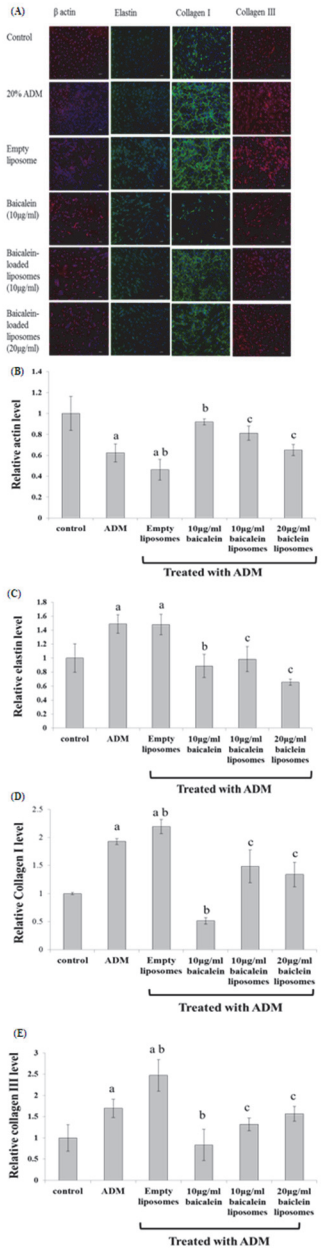


Figure 8. Cytoskeleton and ECM protein expressions by the fluorescent microscopy (A) Immunofluorescence staining of Hs68 fibroblasts were photographed by the microscope and CCD camera system (× 100 magnification). Quantification of fluorescent intensity for Protein expression levels of (B) β-actin, (C) elastin, (D) type I collagen and (E) type III collagen was determined by Image J. a: p<0.05 relative to control, b: p<0.05 relative to cells treated with ADM, c: p<0.05 relative to cells treated with liposomes.