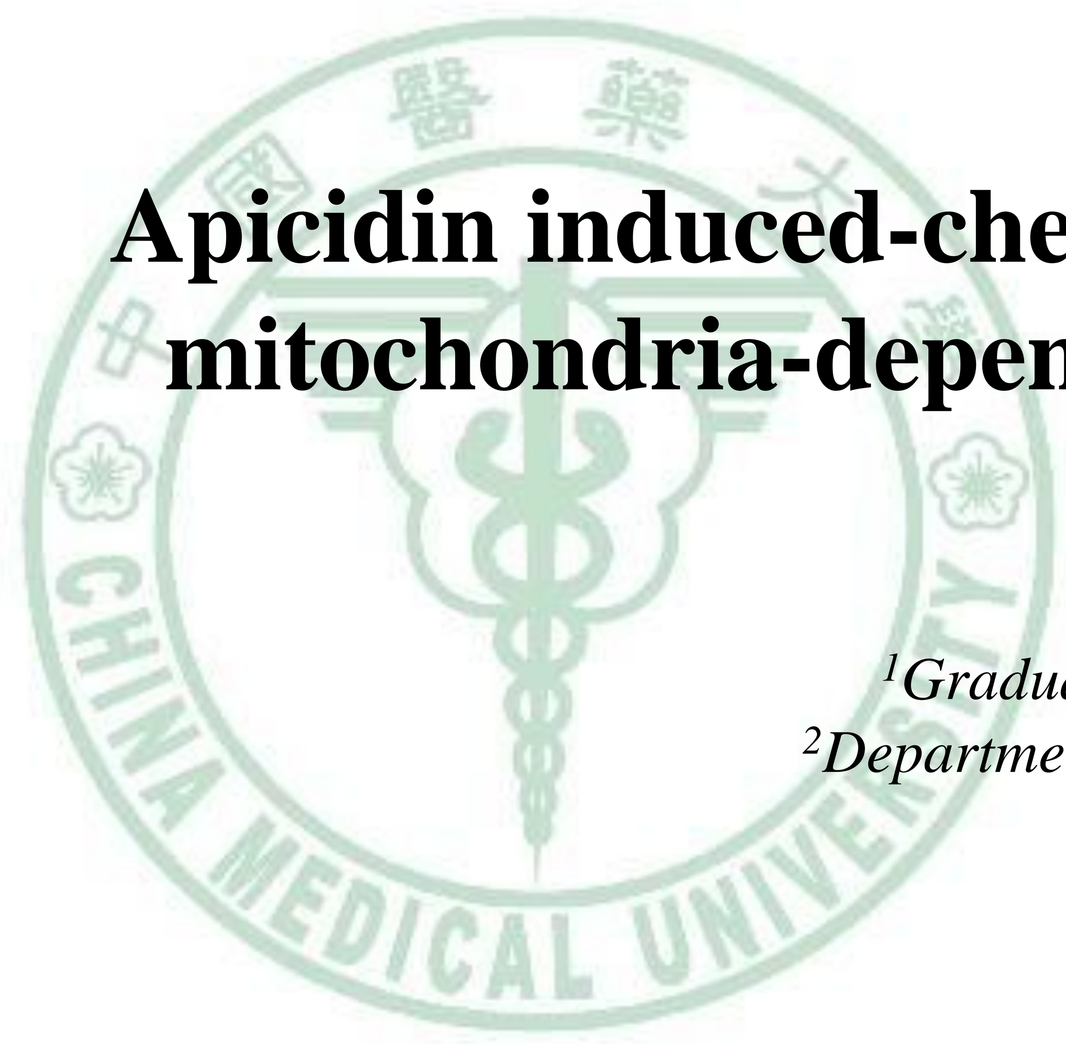


Apicidin induced-chemical resistance is associated with cofilin-1 translocate to mitochondria-dependent apoptosis in HA22T Hepatocellular carcinoma cell

Po-Hsiang Liao[1#] Wei-Wen Kuo[2] Chih Yang Huang[1]*

[1]Graduate Institute of Basic Medical Science, China Medical University, Taichung

[2]Department of Biological Science and Technology, China Medical University, Taichung

Abstract

An increasing trend of incidence in hepatocellular carcinoma (HCC) has been recorded in most developed countries. HCC ranks among the ten most common cancers worldwide. In recent years, use of chemical drugs to treat cancer are very popular, but also induce drug resistance in cancer cells. Apicidin is a novel HDAC inhibitor derived from a fungal metabolite, and it's treatment resistant in HCC remains to be elucidated. To establish a stable liver cancer cell lines chronically resistant to apicidin, HA22T cells were exposed to gradually increasing concentrations of apicidin. In our lab, We observed that Apicidin-resistant (AR) HA22T cells were highly increased in β-catenin nuclear accumulation and significantly decreased in GSK-3-β protein level than HA22T cells, results also showed that AR cells abundantly increased in Tbx3, a downstream target of the Wnt pathway which implicated in liver tumorigenesis metastasis. In addition, we also observed that Apicidin-resistant (AR) HA22T cells were highly increased cofilin-1. Some studies indicated that after induction of apoptosis, cofilin was translocated to mitochondria before release of cytochrome *c* and dephosphorylation of cofilin will promote Bax translocate to mitochondria , It means cofilin may play an important role in mitochondria-dependent apoptosis

Methods and Materials

Cell culture

HA22T was grown in Dulbecco's modified Eagle's medium (DMEM). All mediums were supplemented with 10% fetal bovine serum (FBS), 1% penicillin, and 0.1mM Non-essential amino acids, 1.5g/L sodium bicarbonate and 1.0mM Sodium pyruvate at 37 ˚C in 5% CO_2.

Whole Cell Extract

The cells were extracted in a cell lysis buffer (50mM Tris-base, 0.5M NaCl, 1.0mM EDTA, 1% NP40, 1% Glycerol, 1mM -Mercaptoethanol, Proteinase k inhibitor). The extracts were clarified by centrifugation

Western blotting

An aliquot of each sample equivalent to 40 μg protein was boiled after addition of the appropriate amount of 5x sample buffer. The samples were separated on 10% SDS-polyacrylamide gels (PAGE) and electrophoretically transferred onto nitrocellulose filters using the Bio-Rad electrotransfer system Equal transfer was verified by Ponceau staining of the membranes. Antigen–antibody complexes were visualised with HRP-coupled secondary antibodies and a custom-made ECL detection system .

Reverse-Transcription PCR

The total RNA from cells was isolated and purified with Quick-RNA™ MiniPrep (ZYMO RESEARCH) according to the manufacturer's protocols. One microgram of total RNA from each sample was subjected to first-strand cDNA synthesis using TaqMan reverse transcription reagents kit (TAKARA BIOTECHNOLOGY, Otsu, Japan) in a total volume of 50 μL. Reverse transcription reaction was done at 42℃ for 65 minutes followed by 90℃ for 5 minutes.

Results

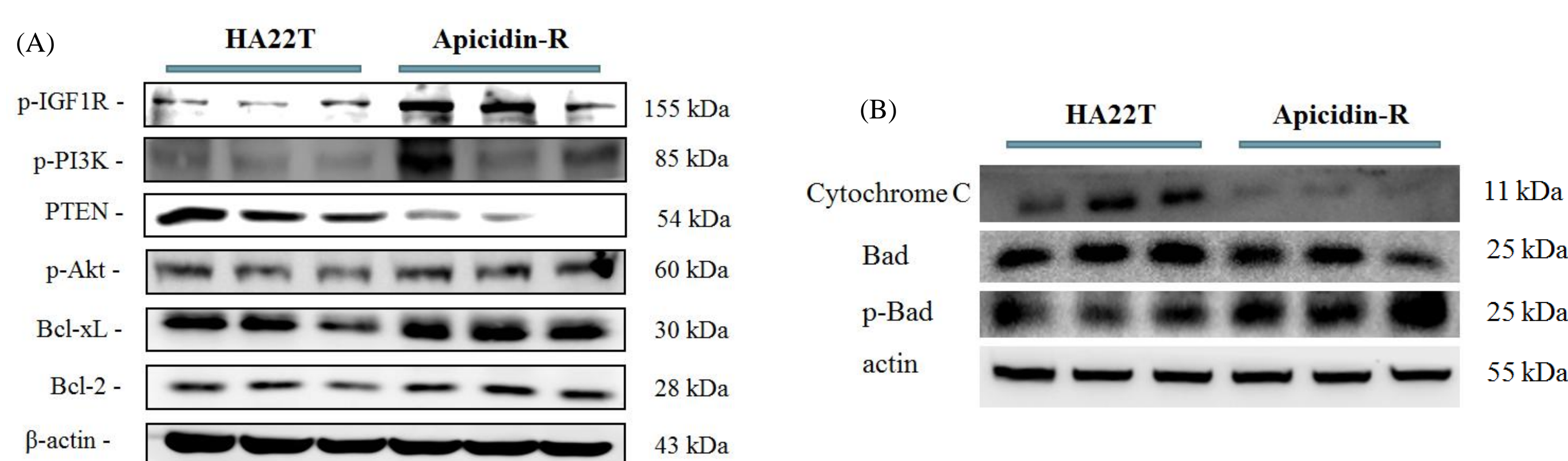
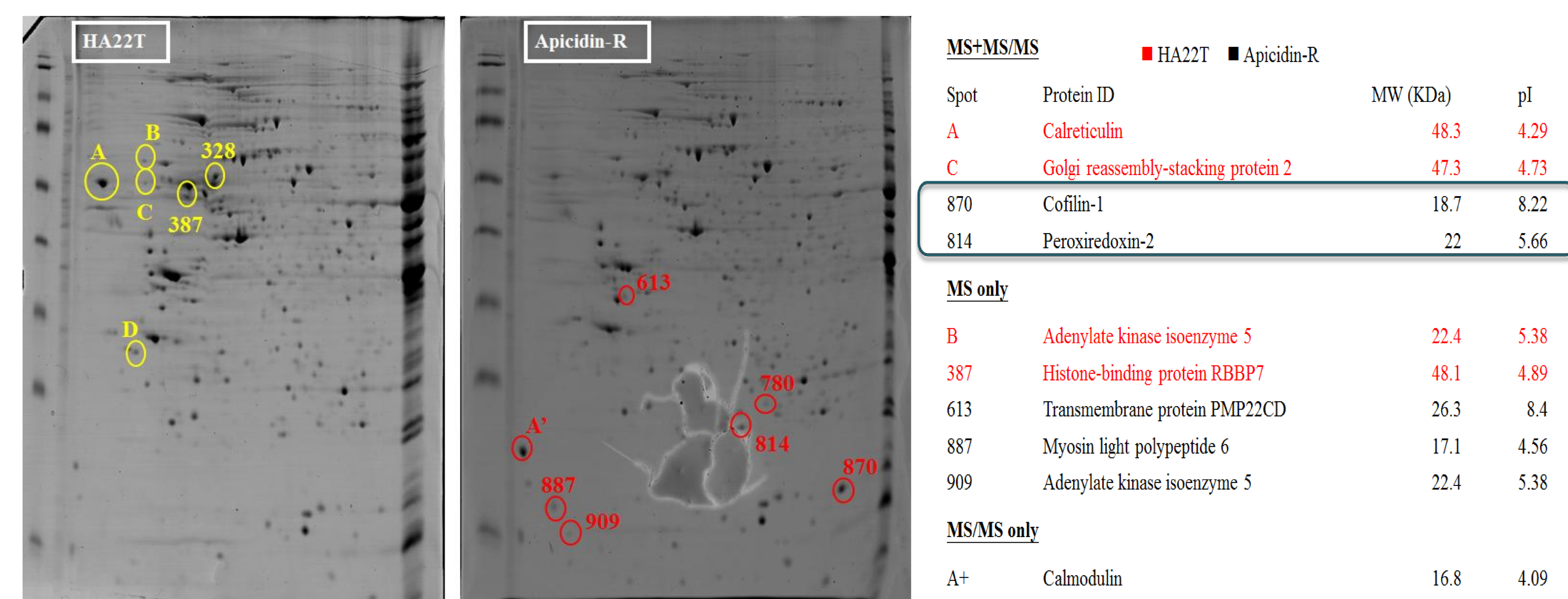


Fig. 1 The apoptosis pathways were both highly suppressed in Apicidin-R HA22T cells. (A)The Western blots were carried out with anti-p-IGF1R, p-PI3K, PTEN, p-Akt, Bcl-xL, Bcl-2 and β-actin antibodies. (B)Western blot analyzed of cytochrome C, Bad and p-Bad in HA22T and Apicidin-R cells.



MS+MS/MS ■ HA22T ■ Apicidin-R

Spot	Protein ID	MW (KDa)	pI
MS+MS/MS			
A	Calreticulin	48.3	4.29
C	Golgi reassembly-stacking protein 2	47.3	4.73
870	Cofilin-1	18.7	8.22
814	Peroxiredoxin-2	22	5.66
MS only			
B	Adenylate kinase isoenzyme 5	22.4	5.38
387	Histone-binding protein RBBP7	48.1	4.89
613	Transmembrane protein PMP22CD	26.3	8.4
887	Myosin light polypeptide 6	17.1	4.56
909	Adenylate kinase isoenzyme 5	22.4	5.38
MS/MS only			
A+	Calmodulin	16.8	4.09

Fig. 2 Two-dimensional gel Electrophoresis and analysis results of HA22T and Apicdin-resistant HA22T Hepatocellular Carcinoma Cells. Crude cell lysates were prepared and separates using the high-resolution 2D gel electrophoresis technique. Proteins with a pI range from 4 to 10 and a molecular weight from 5kD to 170 kD are displayed

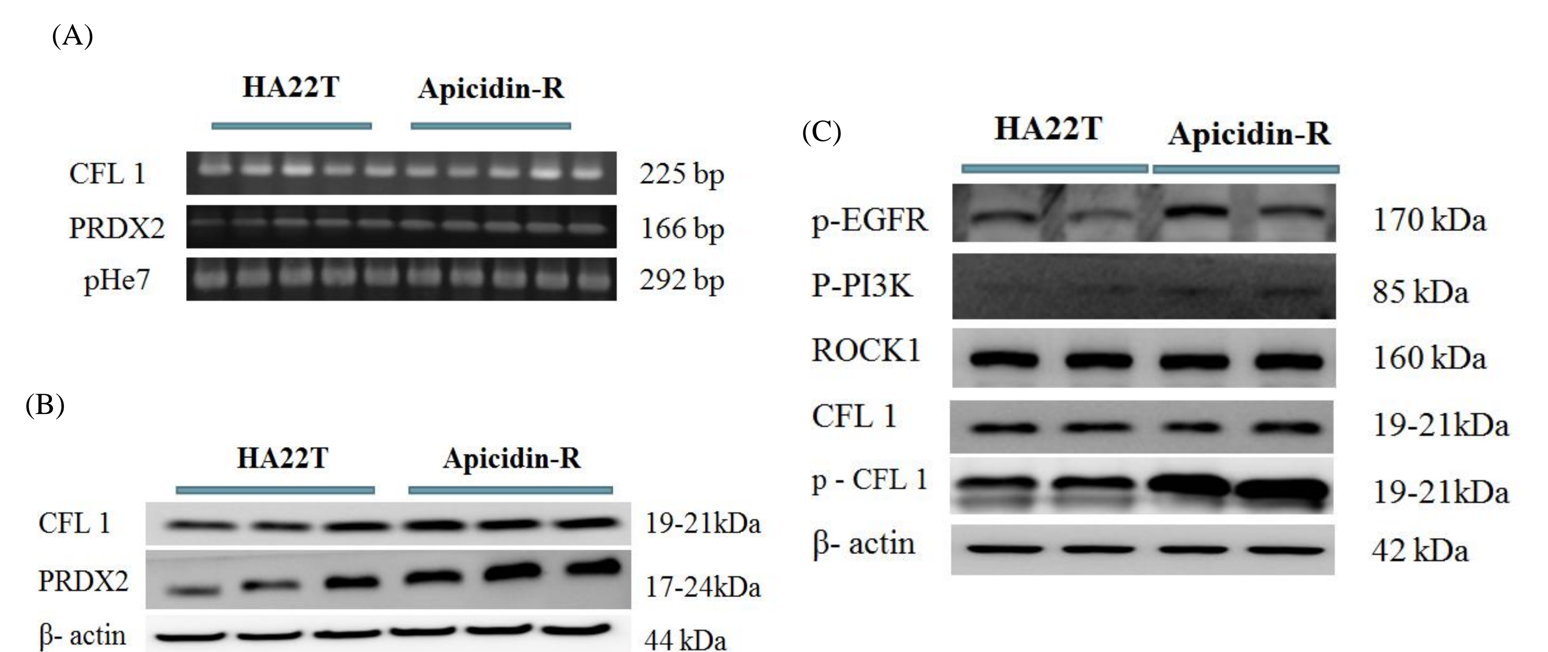


Fig. 3 To investigate the function of cofilin-1 and peroiredoxin-2 between Apicidin-R HA22T cells. (A)The expression of cofilin-1 and peroiredoxin 2 in HA22T and Apicidin-R cells were detected by reverse transcription-PCR. (B) A)The expression of cofilin-1 and peroiredoxin 2 in HA22T and Apicidin-R cells were detected by western blot. (C) The phosphorylation of cofilin-1 in HA22T cell may through EGFR/PI3K/ROCK path way.

Conclusion