Cofilin-1 determines Apicidin-induced chemoresistance in HA22T Hepatocellular carcinoma cell

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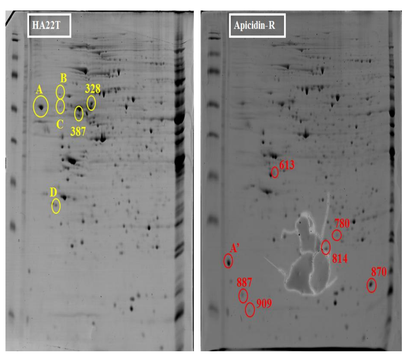
Abstract

Hepatocellular carcinoma (HCC) is one of the most common cancer in the worldwide. Despite of several treatment modes, resistance to chemotherapeutic agents is a major problem in oncology, which limits the effectiveness of anticancer drugs. Therefore to overcome this problem, in the present study we used HDAC inhibitor (Apicidin) to establish stable HA22T resistant cell lines and then we analyzed the molecular mechanism by how this drug induces resistance nature to HA22T cells. Compared with the WT cells, Apicidin resistant cells showed high metastatic ability and prosurvival ability. 2D electrophoresis data showed that, several proteins were altered in Apicidin resistance cells. We chose Cofilin 1 (CFL1) as an important candidate gene, as this was highly expressed in Apicidin resistance cells than the normal cells. Furthermore, other studies indicated that, overexpression of CFL1 or phosphorylation of cofilin doesn't affect tumor metastasis ability. However we also observed that Apicidin-R cells decreased ROS accumulation and mitochondria-dependent apoptotic pathway via phosphorylation of cofilin. All this result indicate the role of cofilin in drug resistance mechanism.

Results

(A)



(B)

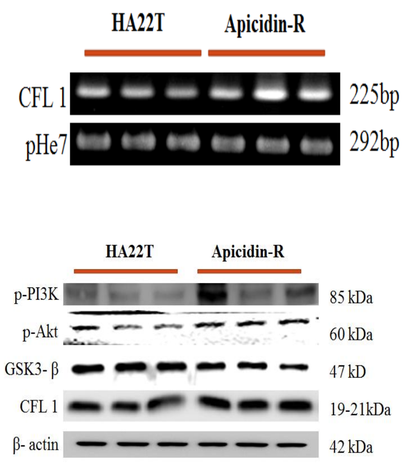
MS+MS/MS ■ HA22T ■ Apicidin-R

Spot	Protein ID	MW (KDa)	pI
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

Figure 1. Different proteins expression between HA22T and Apicidin-R cells

(A) WT and Apicidin-R HA22T cells were analyzed for differential protein expression using 2-DE gel electrophoresis
(B) Analysis of protein spots by MS+MS/MS

(A)



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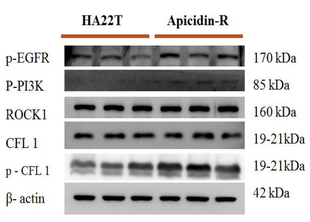


(C)

(D)

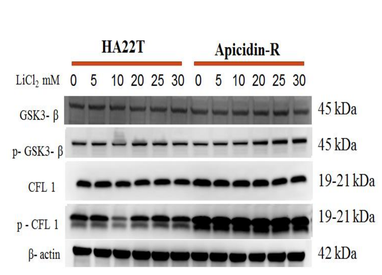
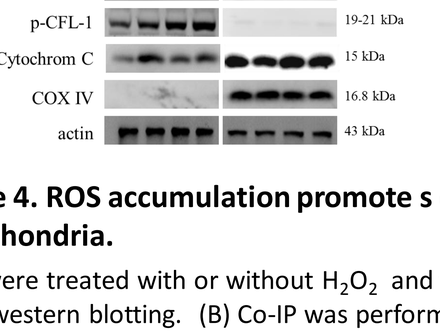


Figure 2. Cofilin phosphorylation in Apicidin-R cell is not via PI3K/Akt/GSK3 and EGFR/ROCK pathway

(A) mRNA expression of Cofilin was analyzed by RT-PCR, in which pHe7 was used as an internal control. Proteins were collected and then analyzed for (B) EGFR/ROCK/CFL pathway; (C) PI3K/Akt/GSK3 pathway (D) Cells were than treated with LiCl2 in a dose dependent manner, and analyzed for GSK3-β and CFL-1 expression using western blotting. β-actin was used as an internal control.

(A)



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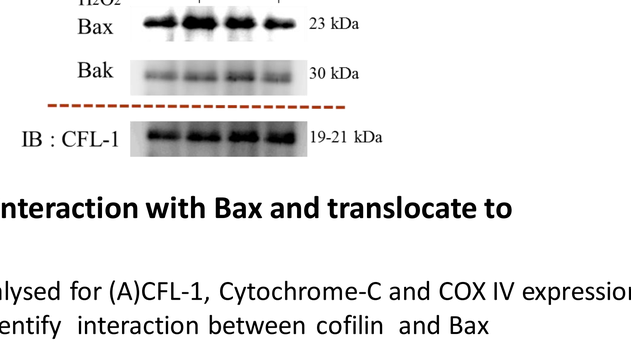


(C)

(D)

Figure 3. Apicidin and H_2O_2 induced ROS accumulation in HA22T and Apicidin-R cells

(A)Cells were treated with various concentration of H_2O_2 and then analysed for ROS accumlation inside the cells. Mitosox dye indicate ROS accumulation in mitochondria and DCF stain indicates total ROS accumulation (B) Quantitative results of figure 3A, (C)Determination of total ROS accumulation by DCF, (D)H_2O_2 decrease cell viability in bothHA22T and Apicidin-R cells was analyzed by MTT assay.

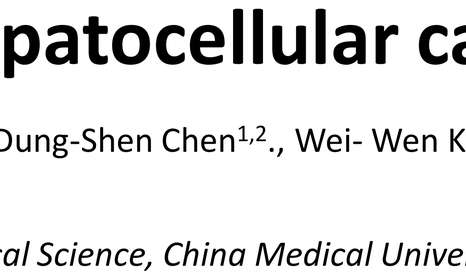
(A)

(B)

Figure 4. ROS accumulation promote s cofilin interaction with Bax and translocate to mitochondria.

Cells were treated with or without H_2O_2 and then analysed for (A)CFL-1, Cytochrome-C and COX IV expression using western blotting. (B) Co-IP was performed to identify interaction between cofilin and Bax

(A)



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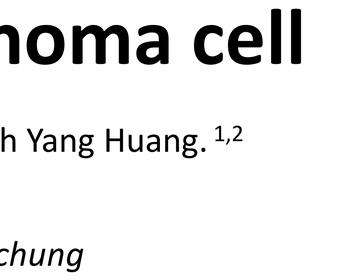

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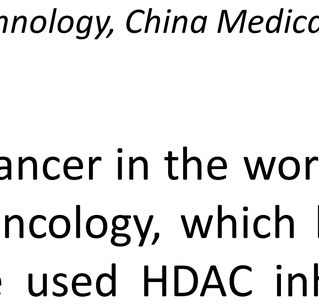

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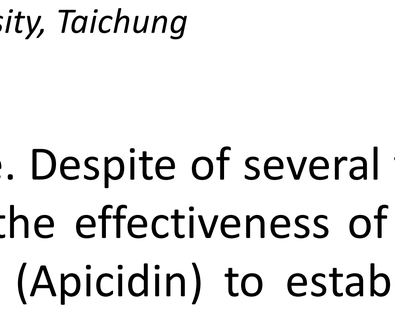

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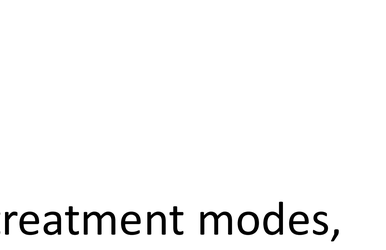
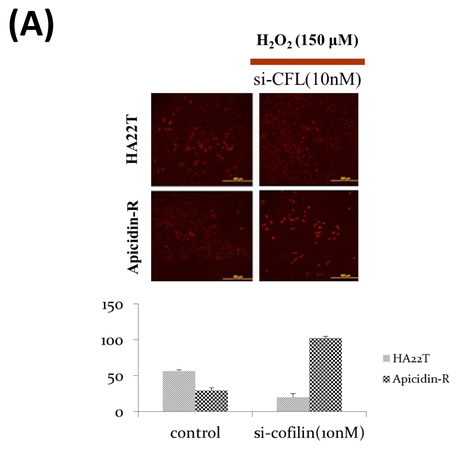


Figure 5. H_2O_2 induce cell apoptosis through promote cofilin translocate to mitochondria

(A) H_2O_2 downregulate cell viability by MTT assay
(B) H_2O_2 promote cofilin phosphorylation in Apicidin-R and Induce cytochrom C expression in HA22T
(C) Overexpression of cofilin and treat H_2O_2 induced cell apoptosis in HA22T cell.
(D) TPA promote cofilin dephosphorylation and cell death
(E) NAR induce cofilin phosphorylation and decrease H_2O_2 damage

(A)



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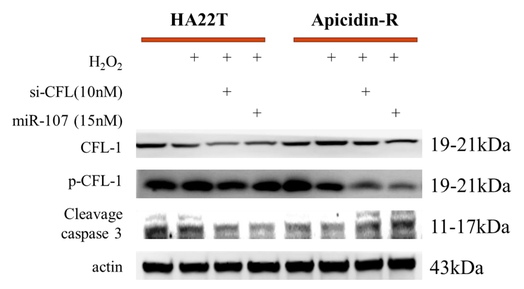
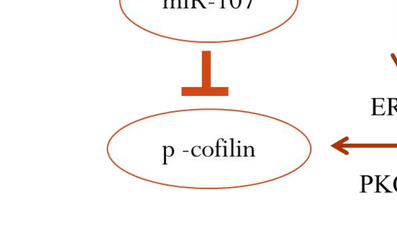


Figure 6. Knockdown cofilin downregulate of H_2O_2 induce cell apoptosis in Apicidin-R cell.

(A) Knockdown cofilin decrease H_2O_2 induced ROS accumlate in mitochondria.
(B) Downregulated damage of H2O2 by silence cofilin.

(A)



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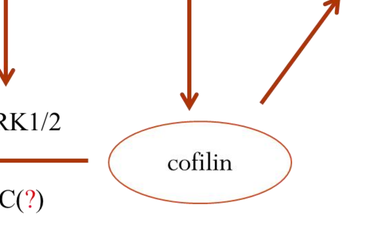

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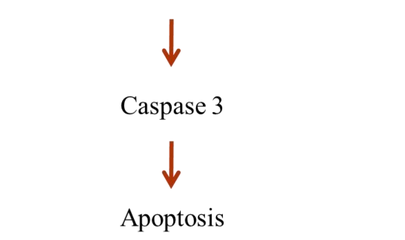

Figure 7. ERK1/2 - the major kinase to phosphorylate cofilin in Apicidin-R cell.

(A) Overexpression of cofilin induce dERK1/2 expression .
(B) H_2O_2 down regulates ERK1/2 and enhace cell apoptosis .
(C) Apicidin induced mitochondria damage by down regulating ERK1/2

Conclusion: