To investigate the role of microRNAs in Apicidin-induced chemical resistance HA22T Hepatocellular carcinoma cells

Po-Hsiang Liao[1]., Wei- Wen Kuo[3]., Chih Yang Huang. [1,2]

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

[2] *Department of Biological technology, Asia University, Taichung*

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

Abstract

Backgrounds:

As of now, chemically synthesized drugs were being used for cancer treatment, and prolonged use of this chemically synthesized drugs make the cancer resistance to treatment and thus result in high mortality rate. miRNAs, a small group of noncoding RNA has drawn a greater attention in the recent years, but their functional role in chemo-resistant HCC cells is not clearly understood. Thus in this present study, we aim to investigate the molecular mechanism altered by miR-449a and miR-122ain both HA22T and Apicidin resistance HA22T (Apicidin-R HA22T) hepatocellular carcinoma cells (HCC).

Methods and Results:

Quantitaive PCR was performed to analyze for the three specific miRNAs, Cell viability and Trypan blue assay was performed to analyze the inhibitory effect of miR449a, Sanger, TargetScan and MicroCosm database & western blotting to identify the putative targets of miR-449a and miR-122a.

Results:

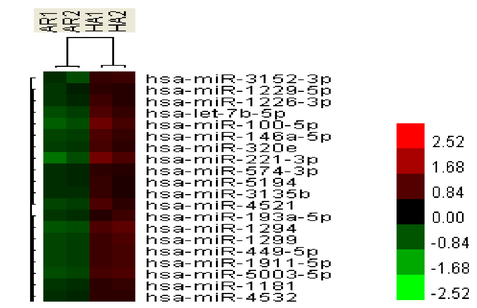
Compared with the HA22T cells, Apicidin resistance HA22T cells expressed lower levels of miR-122a, miR-449a and miR-21a. Overexpression of miR449a decreased the cell viability and cell number in parental and resistance cells. This microRNA was further analyzed for their putative target using miRNA database, we found the Wnt singal pathway, cell cycle protein and c-Met may be targets of miR-449, and then further confirmed by Western blotting assay. Inaddition, miR-449a overexpression decreased Cyclin-D1 expression only in HA22T cells but not in the Apicidin resistance - HA22T cells, and miR-122a overexpression decreased β-catenin and p-Akt expression both in HA22T and Apicidin resistance - HA22T cells.

Results

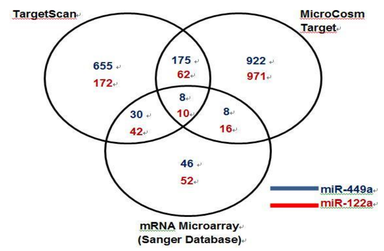
Fig.1

(A)

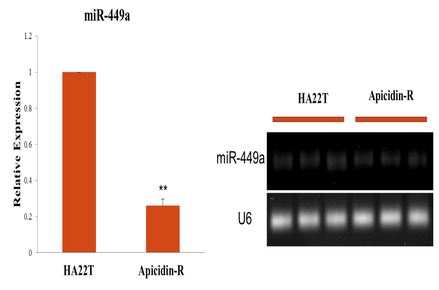
Item	Comparison	up-regulated	down-regulated
1	HA vs. AR	27	317



(B)



(C)



(D)

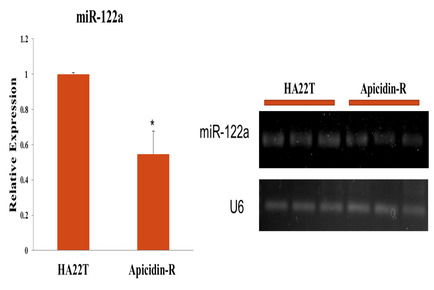
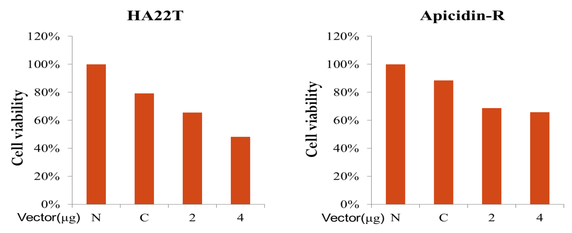


Fig.1. The expression of miR-449a and miR-122a in human HA22T and Apicidin-R HA22T Hepatocellular carcinoma cell. (A)Use microarray to analysis expression of microRNAs (B) Venn diagram of potential targets of miR-449 and miR-122 . The group of genes with decreased expression in mRNA microarray analyses was intersected first with putative targets from the Sanger database.The resulting genes were again intersected with the databases MicroCosm (http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/) and TargetScan. Only 8 genes were predicted by all 3 databases to be targets of miR-449 and 10 genes were predicted by all 3 databases to be targets of miR-122. (C, D) miR-449a and miR-122a expression in was detected by qPCR and by RT-PCR ; U6 was used as an internal control.

Fig.2

(A)



(B)

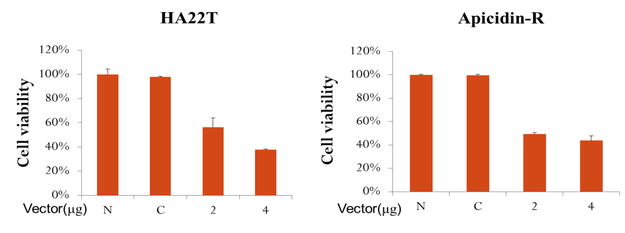
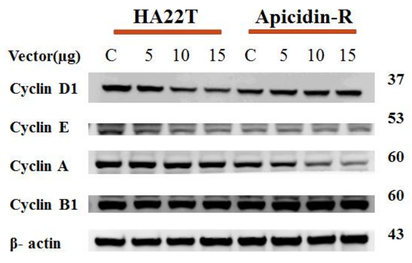


Fig.2. Growth inhibitory effects of miR-449a and miR-122a on hepatocellular carcinoma cell determine by MTT assay. The asterisk indicates the significant difference ($p < 0.01$, Student's t test)(A) The cell growth inhibition of HA22T and Apicidin-R HA22T cells by transfect miR-449a 72hr. (B) The cell growth inhibition of HA22T and Apicidin-R HA22T cells by transfect miR-122a 72hr.(N : normal group., C : control miRNA mimics)

Fig.3

(A)



(B)

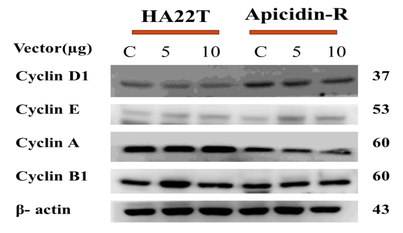
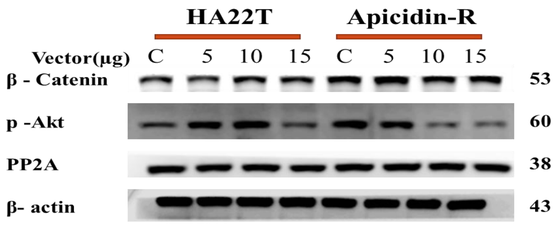


Fig.3. Cell cycle effects of miR-449a and miR-122a on hepatocellular carcinoma cell determine by western blots assay. (A) The cyclin D1 expression will reduce in HA22T but not in Apicidin-R HA22T cells ; cyclin A expression will reduce in Apicidin-R HA22T but not in HA22T cells by transfect miR-449a. (B) There is no difference in cell cycle both in HA22T and Apicidin-R HA22T cells after transfect miR-122a.(C : control miRNA mimics)

Fig.4

(A)



(B)

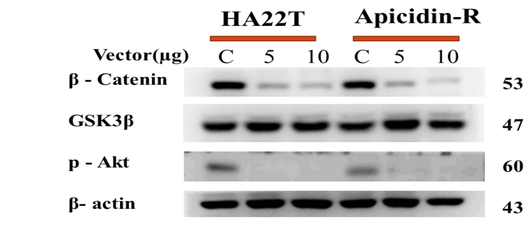


Fig.4 . To search predicted of miR-449a and miR-122a on hepatocellular carcinoma cell determine by western blots assay. (A) β-catenin and p-Akt expression will reduce in Apicidin-R HA22T but not in HA22T cells by transfect miR-449a. (B) β-catenin and p-Akt expression will reduce both in HA22T and Apicidin-R HA22T cells after transfect miR-122a.(C : control miRNA mimics)

Conclusion:

From our experiment we observed that miR-449a and miR-122a were down regulated in HCC cells, and by overexpressing this two miRNAs decreased HA22T and Apicidin resistance - HA22T cell growth may by inhibiting cell cycle and survival protein expression. Using human samples and animal models will be performed to further analyze the functional role of miR449a in HCC.