MicroRNAs-449 REGULATE DOXORUBICIN RESPONSE THROUGH *ACSL4* MODULATION IN TNBC

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Torres S.[1], Garrido-Cano I.[1], Cervera R.[1], Burgués O.[2,4], Hernando C.[3], Martínez M.T.[3], Zazo S.[4,5], Rojo F.[4,5], Bermejo B.[3,4], Lluch A.[1,3,4,6], Cejalvo JM.[1,3,4], Eroles P.[1,4,6], Tormo E. [1,4]

1. Biomedical Research Institute INCLIVA, Valencia, Spain. 2. Pathology Department, Hospital Clínico València, University of Valencia, Spain. 3. Clinical Oncology Department, Hospital Clínico Universitario de Valencia, Valencia, Spain. 4. Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Valencia, Spain. 5. Fundación Jimenez Díaz, Madrid, Spain. 6. Universitat de Valencia, Valencia, Spain

BACKGROUND AND AIMS

Triple negative breast cancer (TNBC) accounts for 10-20% of all breast cancer (BC) cases. The high rate of aggressiveness, relapse and mortality require the search for new therapeutic targets. ACSL4 (Acyl-CoA Long Chain Family Member 4), a key enzyme in the fatty acids' metabolism, has been related to modulation of drug resistance. The hypothesis of this work is that microRNAs-449 family (miRs-449) directly regulates ACSL4, and therefore, could modulate the response to doxorubicin (DOX).

MATHERIALS AND METHODS

ACSL4 mRNA and miRs-449 expression was evaluated in a cohort of TN primary BC patients (n=36) and compared with healthy breast tissues (n=23) from Hospital Clínico de Valencia. TNBC cell lines were used for *in vitro* validation.

Luciferase assay was performed to asses miRs-449/ACSL4 direct interaction

Gene and protein modulation of ACSL4 and its downstream pathways were analyzed by qRT-PCR, WB and functional assays after miRs-449 mimics/inhibitors and ACSL4 siRNA transfection.

RESULTS I

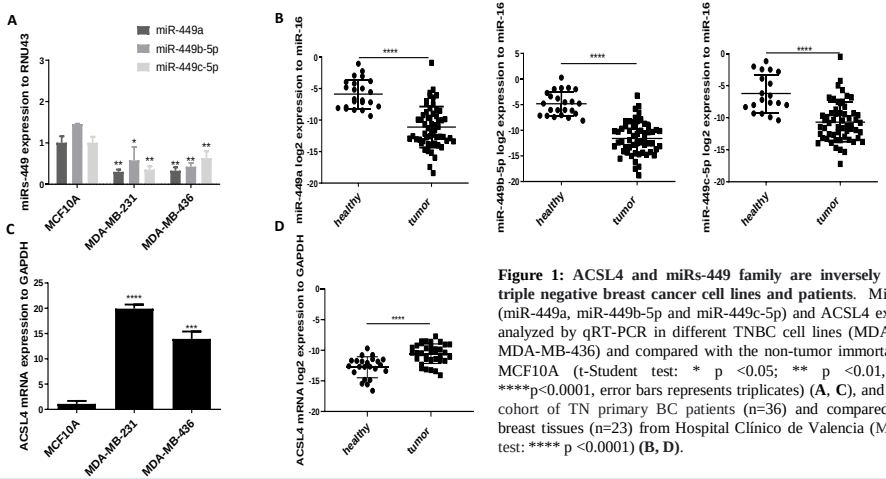

Figure 1: ACSL4 and miRs-449 family are inversely correlated in triple negative breast cancer cell lines and patients. MiRs-449 family (miR-449a, miR-449b-5p and miR-449c-5p) and ACSL4 expression were analyzed by qRT-PCR in different TNBC cell lines (MDA-MB-231 and MDA-MB-436) and compared with the non-tumor immortalized cell line MCF10A (t-Student test: * p <0.05; ** p <0.01, ***p<0.001, ****p<0.0001, error bars represents triplicates) (**A**, **C**), and in a discovery cohort of TN primary BC patients (n=36) and compared with healthy breast tissues (n=23) from Hospital Clínico de Valencia (Mann-Whitney test: **** p <0.0001) (**B, D**).

RESULTS II

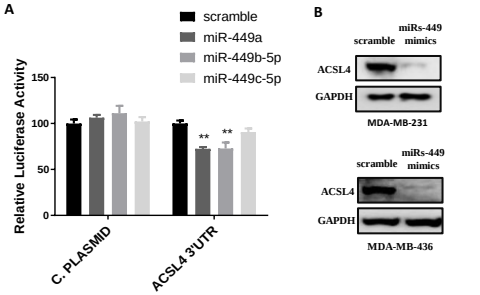

Figure 2: MiRs-449a and b directly bind with 3'UTR of *ACSL4* and inhibit its expression. Luciferase reporter assay was performed in HEK-293T cell line transfected with pEZX-MT06 (3'UTR ACSL4 containing or empty vector), in the presence of miR-449a/b/c mimics. (t-Student: ** p <0.01, error bars represents triplicates) (**A**). ACSL4 was analyzed by western-blot after 72h of transfection with small-interfering RNA (siRNA) targeting *ACSL4* in MDA-MB-231 and MDA-MB-436 cell lines (**B**). C.PLASMID: control plasmid.

RESULTS III

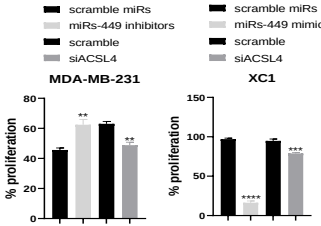

Figure 3: miRs-499 mimics/siACSL4 transfection sensitizes doxorubicin-resistant cell line (XC1) to doxorubicin. Cell proliferation was measured by WST-1 assay after 48h 1uM DOX and cotransfected with 100nM small-interfering RNA (siRNA) targeting *ACSL4* or 50nM miRs-449 inhibitors/mimics, for MDA-MB-231 and XC1 cell line, respectively. Proliferation of untreated cells was set as 100% (t-Student test: ** p <0.01, ***p<0.001, ****p<0.0001, error bars represents triplicates)

RESULTS IV

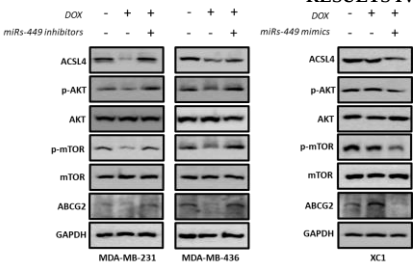

Figure 4: miRs-449 mimics transfection sensitize doxorubicin-resistant cell line (XC1) to doxorubicin through mTOR/ABCG2 axis modulation. ACSL4, mTOR pathway and ABCG2 expression was evaluated by western-blot after 5uM 24h of DOX and cotransfected with miRs-449 inhibitors for MDA-MB-231 and MDA-MB-436 cell lines, or miRs-449 mimics for XC1 cell line. GAPDH was used as a loading control.

RESULTS V

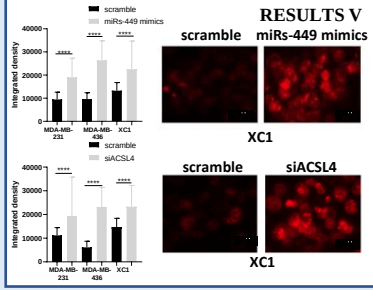

Figure 5: ACSL4 silencing and miRs-449 mimics transfection increases doxorubicin intracelular acumulation. Intracellular DOX accumulation was evaluated by confocal microscopy after 5uM 3h incubation of DOX (red) in MDA-MB-231, MDA-MB-436 and XC1 cell lines transfected with 50 nM of miRs-449 mimics or 100nM small-interfering RNA (siRNA) targeting *ACSL4*. The results are represented as means ± SD of integrated density t-Student: **** p <0.0001. Representative images of XC1 cell line are shown. Scale bar:100 um

CONCLUSIONS

The expression of ACSL4 is higher in TNBC cell lines and patients. Inversely, miRs-449 are downregulated. This study suggests a possible role of miRs-449 family in DOX response through direct ACSL4 repression and mTOR/ABCG2 axis modulation in TNBC

There are no relevant conflicts of interest to disclosure

sand.torres95@gmail.com

Funded by GVA2019/109 and PI18/01219