miRNA-BASED THERAPY TO OVERCOME CHEMORESISTANCE IN CERVIX CANCER

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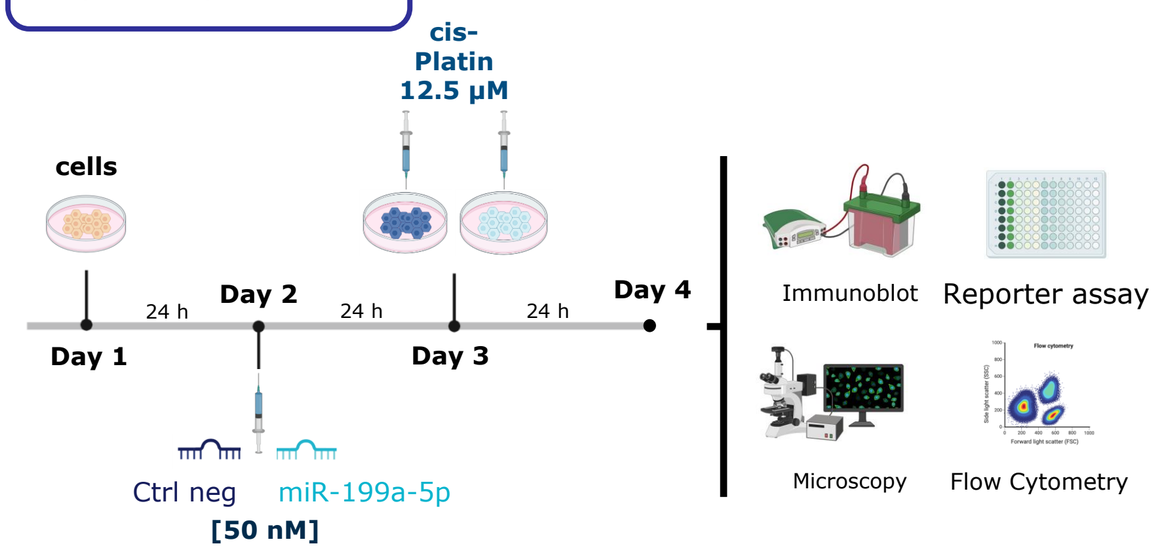
BACKGROUND

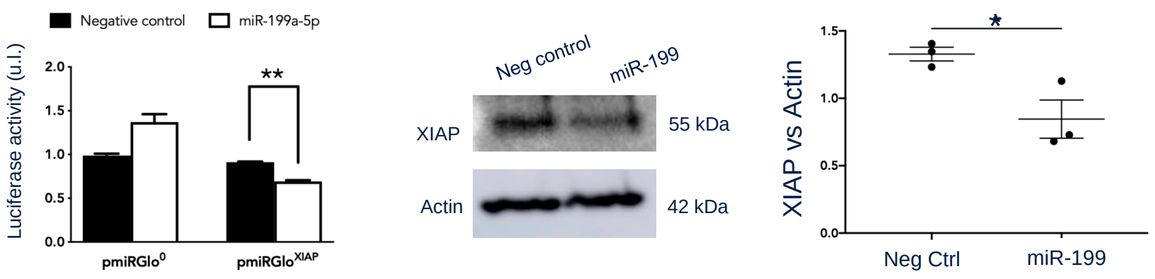
Cervix Cancer (CC) is in the top ten of the **cancer types as the fourth** most frequently **diagnosed** and **the fourth** cancer type in mortality in women worldwide (1). The standard **first-line treatment for CC** is the combination of surgical debulking and chemotherapy with cisplatin or taxane derivatives (2), however tumor cell **chemoresistance** is a major complication for long-term therapeutic success. Besides other molecular mechanisms (3), this resistance is associated, with i) **genetic** factors and ii) **epigenetic** factors, including alterations in microRNA (miRNAs) expression. miRNAs are a class of endogenous and highly conserved noncoding RNAs, of about 18–25 nts in length, that regulate gene expression post-transcriptionally (4, 5). Dysregulated miRNAs in cancer can be divided into two groups: oncogenic miRNAs and oncosuppressor miRNAs (6-9). Both groups are closely related with the risk of cancer progression, metastasis, and therapeutic response to chemo- and radiotherapy resistance (10,11). The high expression and activity of proteins involved in the apoptosis pathway, as **X-linked inhibitor of apoptosis protein (XIAP)**, are key factors in several cancer types, including cervical cancer (12-16). XIAP upregulation allows tumor cells to escape apoptosis chemotherapy- induced, and its elevated expression in cancer contribute for the resistance of tumor cells to conventional therapeutic treatments. Intense efforts are being made to reduce XIAP levels, and one of the most recent strategy is the use of miRNAs targeting XIAP, as cancer therapy (17, 18). In the present work, for the first time we show that **miR-199a-5p negatively regulates XIAP expression** in C6 (glioma), Neuro-2A (neuroblastoma) and HeLa (cervical cancer) cell lines (19). **Our results show that treatment with miR-199a sensitizes HeLa cells to cisplatin-induced cell death, due to a negative regulation on XIAP expression.**

Work Flow Chart

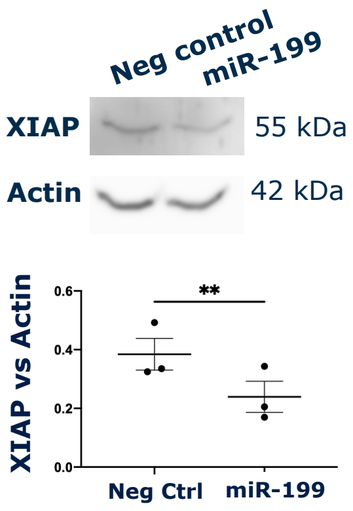


miR-199 reduces the XIAP protein level by directly targeting XIAP 3'-UTR

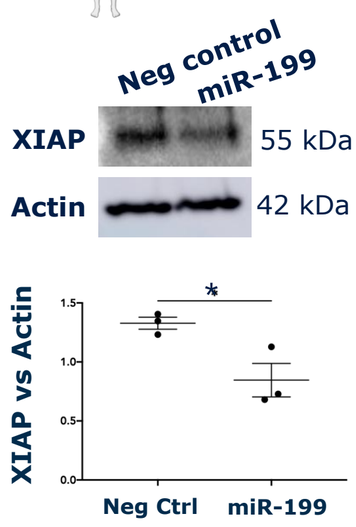
A. C6 cell line



B. N2a cell line



C. HeLa cell line



In panel **A,** we validated the molecular and functional binding of miR-199a-5p mimetic to the 3'-UTR of the mRNA for XIAP protein (3'-UTR-XIAP) in rat C6 cell line, using the Dual-Luciferase Reporter Assay System kit (Promega). After 24h of transfection, we measured the ratio of luciferase/renilla activity and normalized it to the double negative condition (co-transfection with pmiR-Glo0 + negative control mimetic). The presence of the miR-199a-5p mimetic significantly reduced the luciferase activity compared to cells transfected with the negative control mimetic (**p< 0.01 in paired t-test; Bars represent mean±SEM from 3 independent experiments). Then, we evaluated functional effect of miR-199a-5p mimetic transfection on XIAP expression in C6 cells protein extracts. Densitometric analysis of XIAP expression levels relativized to α-actin. Representative immunoblot images and dot-plot show a **significant reduction of XIAP levels after miR-199a-5p mimetic transfection** compared to negative control mimetic (*p<0.05 in paired t-test; dot-plot represents mean±SEM from n=3 independent experiments) in mouse Neuro-2A (**B**) and human HeLa (**C**) cell lines respectively.

CONCLUSION

Treatment with miR-199a sensitizes HeLa cells to cisplatin-induced cell death, due to a negative regulation on XIAP expression.

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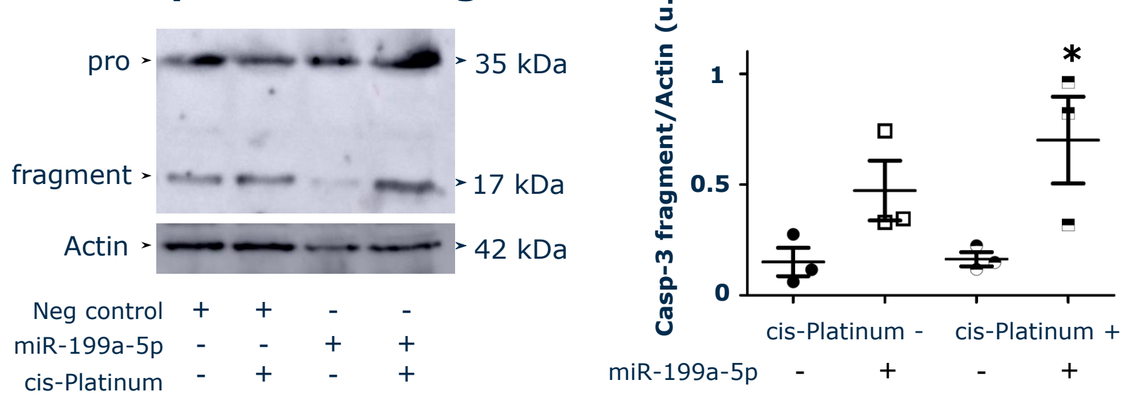
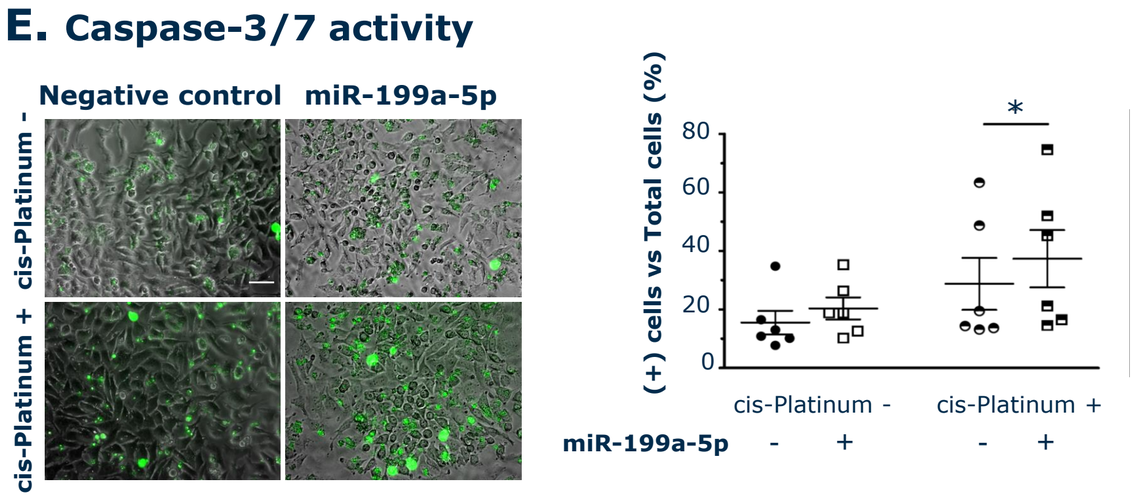
miR-199 increases the sensitivity of cervical cancer cells to cisplatin-induced apoptosis

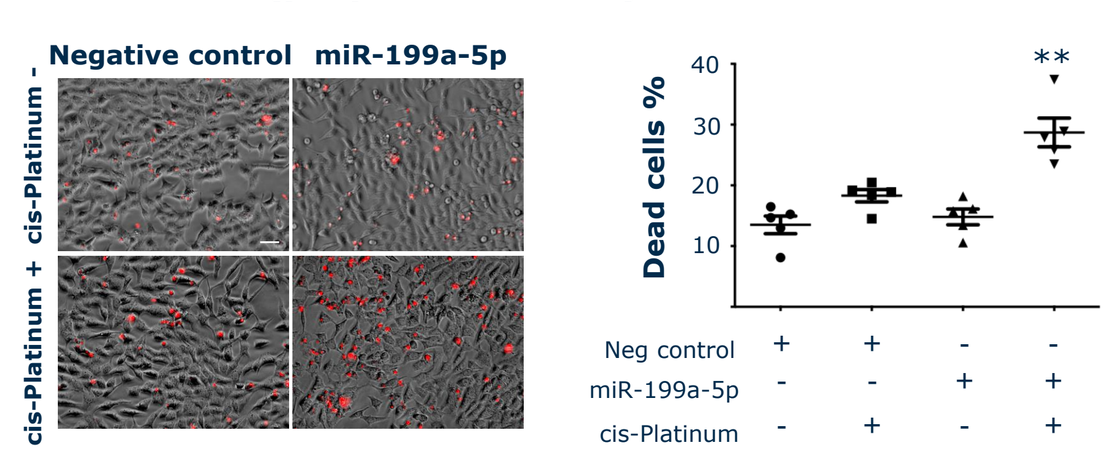
D. Caspase-3 cleavage



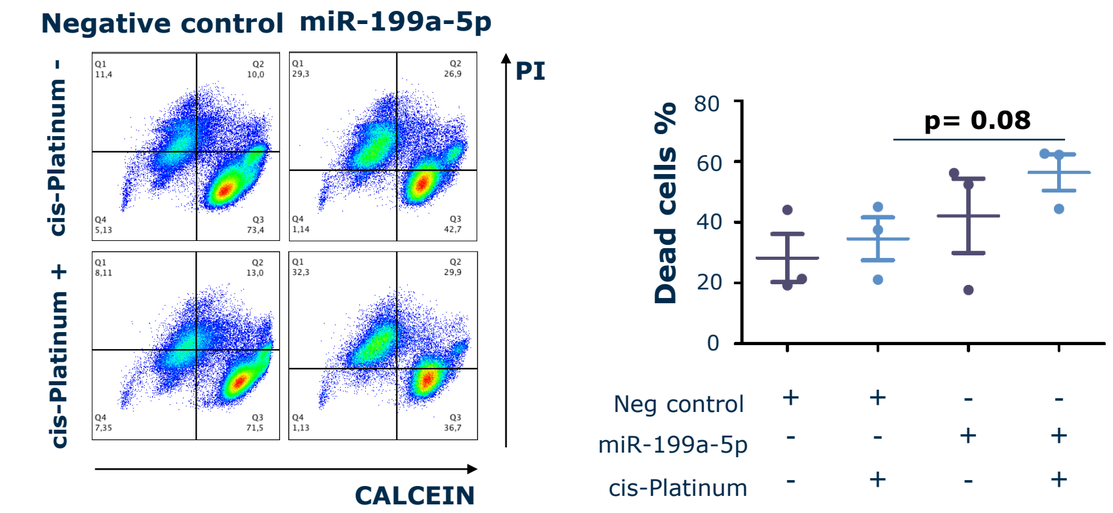
E. Caspase-3/7 activity



F. Cell death (propidium iodure)



G. Cell death (flow cytometry)



Representative immunoblot (**D**) for detection of pro-caspase-3 (35 KDa) and the Caspase 3-cleaved fragment (17 KDa) in protein extracts from HeLa cells. Densitometric values were relativized to α-actin expression. Dot plot summary shows that **miR-199-5p mimetic transfection significantly increased caspase-3 cleavage** (Two-way ANOVA: F_8=11.96; p=0.0086), compared to negative control mimetic (dot-plot represents mean±SEM from n=3 independent experiments). (**E**) Representative images of caspase 3/7 enzymatic activity in HeLa cells using CellEvent™ caspase-3/7 green detection kit (Thermo-Fisher) and acquired by an epifluorescence microscope (DM5000B; Leica Microsystem GmbH). Dot plot summary shows that, after cisplatin treatment, transfection with the **miR-199-5p mimetic significantly increased the percentage of cells with active caspase-3/7**, compared to the negative control mimetic (*p<0.05 in paired t-test; dot-plot represents mean±SEM from n=6 independent experiments). (**F**) Images of HeLa cell to evaluate cell death using propidium iodide staining assay. Dot plot shows that **miR-199-5p mimetic transfection significantly increased the percentage of propidium iodide-labelled cells** compared to those transfected with the negative control mimetic (effect of miR-199-5p mimetic: F_1=13.23 in two-way ANOVA: p=0.002). It also produced a significant sensitization to cisplatin treatment (**p=0.006 in paired t-test relative to treated and negative control cells; n=5 independent experiments). (**G**) Representative plots of the cell distribution populations evaluating HeLa cell death measured by flow cytometry using propidium iodide (dead cells in y-axis) and calcein-AM staining (live cells in x-axis). Dot plot shows that **miR-199-5p mimetic might sensitize cells to cisplatin-induced death** compared to transfection with the negative control mimetic (p=0.08; dot plot represents mean ±SEM from n=5 independent experiments).