Dexamethasone-induced microRNA regulation for pancreatic cancer progression

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DEUTSCHES KREBSFORSCHUNGSZENTRUM IN DER HELMHOLTZ-GEMEINSCHAFT

50 Jahre – Forschen für ein Leben ohne Krebs

Introduction : Pancreatic cancer is one of the leading causes of cancer-related mortality worldwide and is highly therapy-resistant to standard chemotherapy including gemcitabine. Glucocorticoids like dexamethasone (DEX) are often co-administered to reduce inflammation and side effects of tumor growth and therapy. Our group previously showed DEX to be a potent stimulator of epithelial to mesenchymal transition (EMT), cancer progression and metastasis, but the underlying mechanisms were not completely understood. MicroRNAs are a group of small non-coding RNAs that post transcriptionally regulate gene expression. They have been shown to affect several processes, including cancer. In this study, we evaluated the effect of DEX on the microRNA expression profile of pancreatic cancer cell lines with the aim of finding out if miRNAs play a role in modulating the DEX-induced phenotype.

Material and Methods : RNA was isolated at different time points after dexamethasone treatment. RNA was then labeled and hybridized to the Illumina Human miRNA Microarray (Release 21). miRNA data was analyzed using the LIMMA protocol. Differentially expressed miRNAs were validated by RT-PCR. Potential targets of the selected miRNAs were identified *in silico* using TargetScan. Interaction of the miRNA with the 3'UTR of the predicted miRNAs was confirmed with 3'UTR reporter gene assays, and target site binding specificity by site-directed mutagenesis. The effect of miRNA expression on target expression was evaluated by qRT-PCR and Western blotting for mRNA and protein expression, respectively. Functionally, migration was assessed using the wound healing assay, and the ability of single cells to form colonies with the colony formation assay. In vivo experiments were performed with the chick embryo model. ASANPACA cells transfected with 50nM miR-XYZ and treated with 1µM DEX, transplanted on day 9 into eggs, injected IV on day 14 with mimics and lipofectamin to maintain miRNA expression.

1

DEX induces miRNA expression

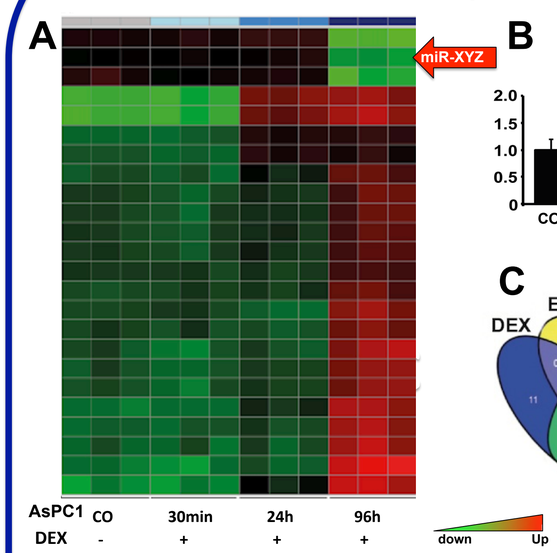

Figure 1 : DEX suppresses miR-XYZ expression. **(A)** Heatmap representation of top regulated miRNAs in DEX- treated AsPC1 cells. Red indicates miRNAs up-regulation induced by dexamethasone treatment, and green indicates down-regulation (three independent biological replicates, $p < 0.05$). **(B)** miRNA microarray results were validated by quantitative PCR analysis as well as miR-XYZ. (**C)** Gene ontology analysis results 7 miRNAs influence in imporant signaling pathways and processes.

2

miR-XYZ directly targets TGFß-2

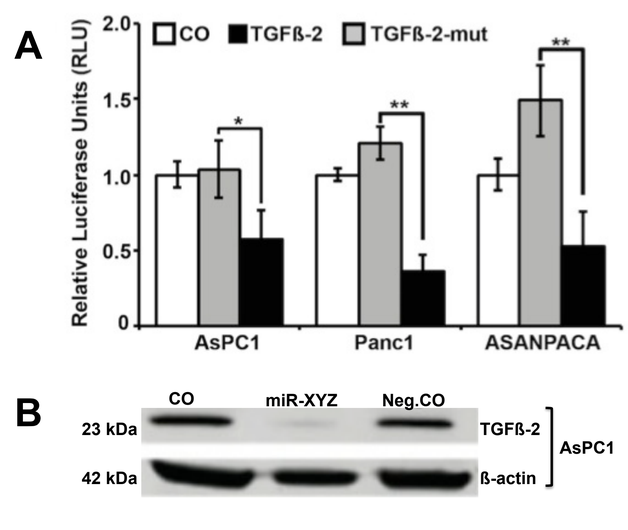

Figure 2. TGFß-2 is a direct target for miR-XYZ **(A)** Luciferase reporter assay and site-directed mutagenesis confirms that miR-XYZ directly targets TGFß-2 through its 3'UTR **(B)** Western blot analysis demonstrates that miR-XYZ repress the protein expression of TGFbß-2.

3

Enhanced expression of miR-XYZ suppresses migration, and colony formation

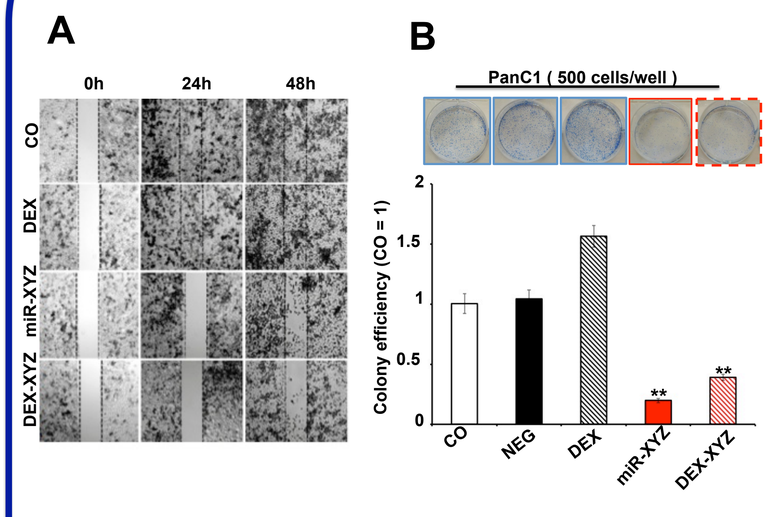

Figure 3: miR-XYZ inhibits migration and colony formation. **(A)**The migration of the PanC1 cells and DEX treated PanC1 cells were found to be suppressed by miR-XYZ. Wound closure was measured and normalized to the negative control. **(B)** Colony formation reveals the effect of the miR-XYZ on cellular survival, colonies are shown in blue staining. The DEX-induced PanC1 cells formed colonies whereas miR-XYZ inhibited this process in control and in DEX-treated cells.

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miR-XYZ reverses EMT *in vitro*

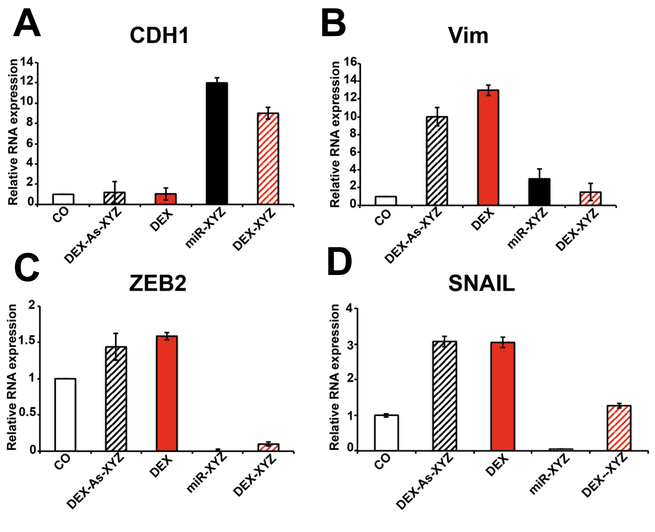

Figure 4 : qRT-PCR demonstrates that miR-XYZ re-activates E.cadherin RNA expression and represses the expression of mesnchymal markers Vimentin, SNAIL and ZEB2 . **(A)** E.cadherin (CDH1) the epthelial marker. **(B)** Vimentin (Vim) is a mesenchymal marker. **(C)** Zinc finger enhancer E.box binding homebox (ZEB2) is a repressor of epithelial proteins. **(D)** SNAIL is a transcription factor and a repressor of E-cadherin expression.

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miR-XYZ reduces tumor size *in vivo* and suppresses proliferation

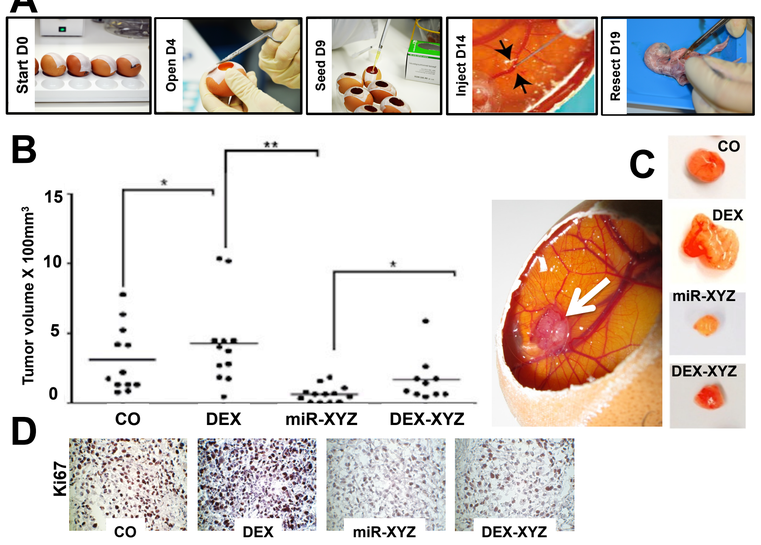

Figure 5:. The fertilized chick egg model was used to study the effect of miR-XYZ on tumor size and proliferation.**(A)**The experiment workflow.**(B)** Graphical illustration of the obtained tumor volume **(C)** Tumors from miR-XYZ and DEX-XYZ were significantly smaller than those from CO and DEX **(D)** Proliferation marker (Ki67) showed significantly lower levels of Ki67 in miR-XYZ than DEX and Control.

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miR-XYZ reverses EMT and targets TGFß-2 *in vivo*

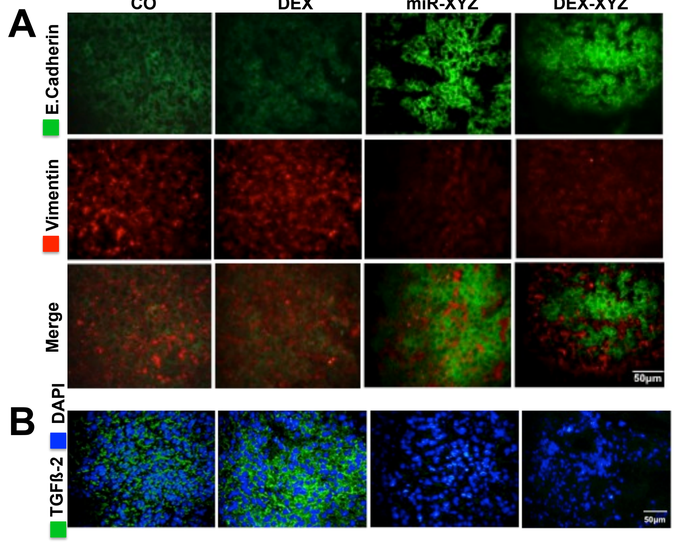

Figure 6: Expression of progression markers were analyzed by immunostaining and fluorescence microscopy for the expression of the E.cadherin, Vimentin and TGFß-2. **(A)** reveals that miR-XYZ strongly re-activate the expression of the epthelial marker E.cadherin aslo with DEX combination. **(B)** shows that miR-XYZ down regulates the expression of *TGFß-2 in vivo, DAPI is a* nucleus marker .

Conclusion: miR-XYZ is the most significantly DEX deregulated miRNA in pancreatic cancer cell lines and targets key members of the TGF-beta pathway, specifically TGFb-2. miR-XYZ significantly reduced proliferation, migration and colony formation of pancreatic cancer cells *in vitro* and *in vivo*, it reduced tumor xenograft growth. miR-XYZ is a potential tumor suppressor miRNA in pancreatic cancer.

Outlook: We are working on assessing the expression of miR-XYZ in patient tissues and identifying the impact of miR-XYZ on drug resistance. Furthermore we are working towards confirming our *in vitro* findings in a mouse model.

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