Combination of miRNA-379-5p and miRNA-125-3p for the Treatment of Platinum-Resistant Ovarian Cancer

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Background

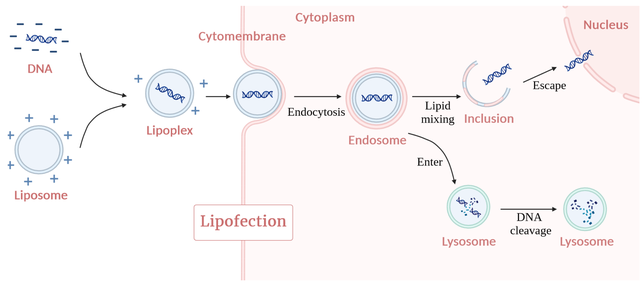
- Approximately 21,000 new cases and 13,000 deaths from ovarian cancer are reported annually, with 73% diagnosed at advanced stages. [1]
- Ovarian cancer is the fifth leading cause of cancer mortality among women in the United States. [2]
- Platinum-resistant ovarian cancer (PROC) is characterized by a decreased sensitivity to platinum-based chemotherapy via upregulated DNA repair pathways. [3]
- Approximately 70% of ovarian cancer patients experience recurrence, with many progressing to platinum-resistant disease. [4]
- Currently, there is no curative treatment for recurrent ovarian cancer, and therapeutic options for PROC remain limited [3]
- MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression and are involved in key cellular processes.[5]
- miRNA-379-5p acts as a tumor suppressor by targeting focal adhesion kinases (FAK) and is downregulated in ovarian cancer samples.
- miRNA-125a-3p inhibits cell invasion, migration, and proliferation while inducing apoptosis and cell cycle arrest in ovarian cancer cells.

Objective: To investigate the dual delivery of miRNA-379-5p and miRNA-125a-3p as a potential treatment strategy for PROC.

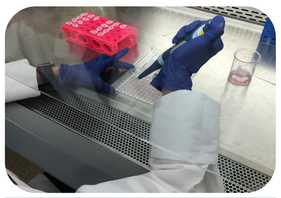
Hypothesis: The combined delivery of miRNA-379-5p and miRNA-125a-3p will reduce cell viability in PROC cells more effectively than either miRNA alone.

Methods

Lipid-Based Transfection



Cell Viability Assay



Addition of MTT reagent
OVCAR3 cells (10,000 cells/well)



Spectrophotometer
Absorbance is read at 570 nm after 3-hour incubation.

Results

Cell Viability Assay

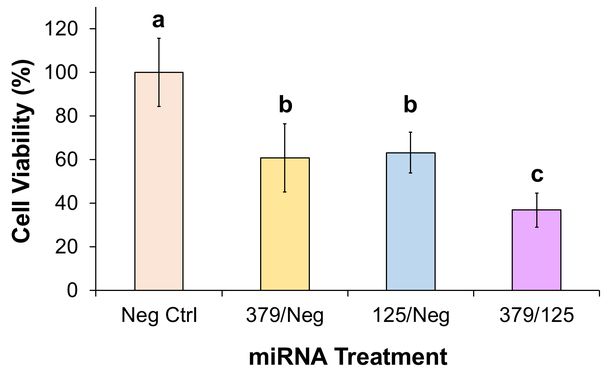


Figure 1. Cell viability of OVCAR-3 cells following transfection with miRNA-379-5p and miRNA-125a-3p. Results are demonstrated as mean ± standard deviation for n=6. Groups with different lowercase letters (i.e. a vs. b vs. c) are significantly different ($p < 0.05$) from one another.

Protein Markers for Western Blot Analysis

Table 1. Western blot markers used to assess apoptosis, signaling and EMT in OVCAR3 cells.

Marker	Type	Function
Actin	Loading Control Protein	Stable structural protein; confirms equal protein loading
PARP	Apoptosis Substrate	Cleaved by caspase-3; indicates apoptosis
Caspase 3	Apoptosis Executioner	Activated in apoptosis; cleaves PARP
LC3B	Autophagy Marker	Forms autophagosome formation; LC3-II indicates autophagy
Akt (pan)	Total Survival Kinase	Reflects total Akt levels; indicates pathway presence
Phospho-Akt (Ser473)	Activated Survival Kinase	Active Akt; indicates an increased survival signaling, reduced by miRNA-379-5p via FAK/AKT inhibition [6]
Phospho-Akt (Thr308)	Activated Survival Kinase	Akt pathway activation; reduced through miRNA-379-5p inhibition of upstream FAK signaling [6]
p44/42 MAPK (ERK1/2)	Total Proliferation Kinase	Reflects ERK levels; indicates proliferation signaling
Phospho-p44/42 MAPK	Activated Proliferation Kinase	Active ERK signaling; promotes proliferation, reduced by miRNA-125a-3p via MAPK regulation [7]
E-Cadherin	Epithelial Marker	Maintains epithelial phenotype; increased expression indicated miRNA-379-5p mediated EMT suppression [6]
Vimentin	Mesenchymal Cytoskeleton Protein	Mesenchymal marker; decreased expression reflects miRNA-379-5p inhibition of EMT and invasion [6]
Snail	EMT Transcription Factor	EMT regulator; reduced expression indicated miRNA-379-5p mediated EMT suppression [6]
MMP-2	ECM Degrading Enzyme	Degrades ECM; reduced activity associated with decreased invasion following EMT suppression
MMP-9	ECM Degrading Enzyme	Degrades ECM; indicates metastatic potential

Discussion

Cell Viability Assay (Figure 1)

- Delivery of miRNA-379-5p and miRNA-125-3p reduced cell viability in OVCAR-3 cells compared to the negative control.
- Both miRNA-379-5p and miRNA-125-3p alone decreased cell viability, showing their individual ability to inhibit cell proliferation.
- Combination delivery showed a significantly greater reduction in viability compared to either miRNA alone.
- These results suggest an enhanced effect of combined miRNA delivery, potentially improving the inhibition of cell proliferation and survival.

Protein Markers for Western Blot Analysis (Table 1)

- Selected protein markers represent pathways in OC including apoptosis, survival signaling (Akt/ERK) and epithelial-mesenchymal transition (EMT).
- Apoptotic proteins (caspase-3, PARP) indicate activation of programmed cell death, while Akt and ERK signaling proteins reflect changes in cell survival and proliferation.
- EMT and invasion markers (E-cadherin, Snail, Vimentin, MMP-2/9) assess changes in cell adhesion and motility with metastatic potential.
- These markers indicate how treatment influences tumor progression and metastatic potential in OVCAR-3 cells.

Conclusions & Future Works

- Initial results show that treatment with miRNA-379-5p and miRNA-125a-3p reduces cell viability in OVCAR-3 cells, with the combination treatment producing the strongest inhibitory effect.
- These results suggest an enhanced effect of combined miRNA therapy in reducing tumor cell growth and proliferation.
- Immunoblotting analysis will be used to evaluate changes in protein expression related to apoptosis, survival signaling, and EMT to better understand the mechanisms underlying these effects.
- Future studies will evaluate newly developed polymer-based transfection as an alternative method to reduce cytotoxicity.

References

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Acknowledgements

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