

The Role of Exosomal miR-181c-3p Within the Ovarian Tumor Microenvironment

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Introduction

Every year, 21,000 people are diagnosed with epithelial ovarian cancer (EOC) and 14,000 die from it [1]. Most patients diagnosed with EOC will be short term survivors (STS) who live for less than two years after treatment and few will be long-term survivors (LTS), who survive over seven years after treatment, creating an urgent need for further research.

MicroRNA (miR) provides a good opportunity to better understand the biological factors behind survival rates because it is a master regulator of gene expression. MicroRNAs that exist in exosomes are transported between different types of cells and can be used to better understand the tumor microenvironment (TME). Previous research utilized microRNA sequencing analysis on formalin-fixed paraffin-embedded (FFPE) tumor tissue samples from LTS and STS of high-grade serous ovarian cancer (HGSC) and found 50 different miRs which are more highly expression in LTS than STS. Analysis of the exosomes isolated from nine HGSC ovarian cancer cell lines indicated that miR-181c-3p is the only exosomal microRNA which is also expressed more in LTS than STS. This miR inhibits angiogenesis and increases endothelial cell apoptosis. Target prediction algorithms have found that leukemia inhibitory factor (LIF) is a target of this miR. Increased LIF expression decreases the trafficking and activation of CD8⁺ cytotoxic T cells (CTLs). These activated cells have a positive impact on survival times and have been proven to be more prevalent in STS than LTS. We hypothesize that miR-181c-3p can be transported amongst various cell types within the TME to suppress LIF expression, increasing CD8⁺ T cell activation and trafficking.

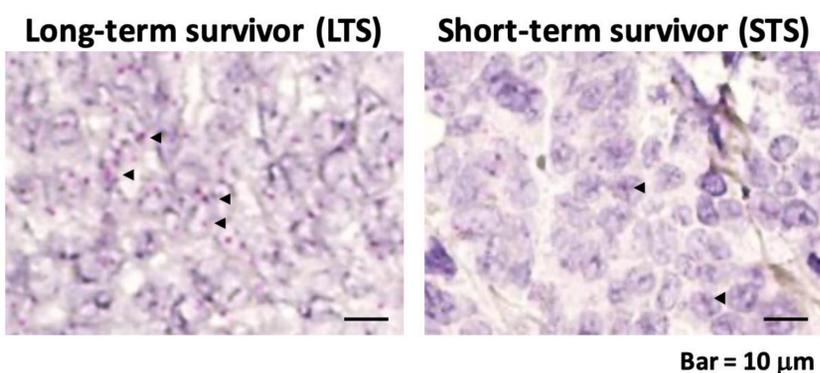


Figure 1: Colorimetric staining indicates that LTS sample is more densely populated with miR-181c-3p than STS

Methods

1. miR181c-3p was stained in FFPE tumor samples from LTS and STS using miRNAscope assay (Advanced Cell Diagnostics, Inc.)
2. miR-181c-3p expression in patient samples measured using qRT-PCR analysis
3. Correlation analysis between miR-181c-3p expression and intratumoral CD8⁺ T cell count in patient samples.
4. PEO1 and PEA1 cell lines had both demonstrated high levels of miR-181c-3p, so they were treated with a control and miR-181c-3p inhibitor. qRT-PCR and Western Blot analysis were used to compare the LIF mRNA expression in those cells.

Results

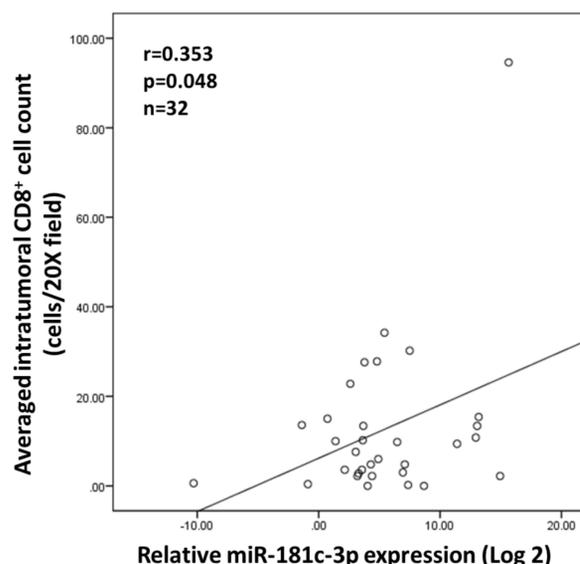


Figure 2: Correlation analysis of qPCR results and number of CD8⁺ cells indicate positive correlation between miR-181c-3p and number of CD8⁺ cells.

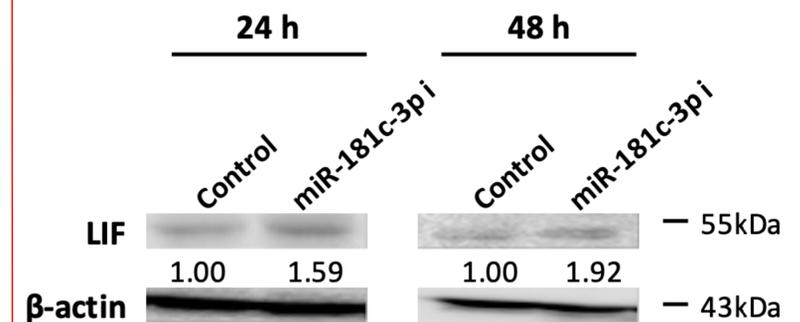


Figure 3: Western Blot results on PEO1 cell line indicates that LIF expression is higher in cells that are treated with miR-181c-3p inhibitor than those treated with a β-actin loading control.

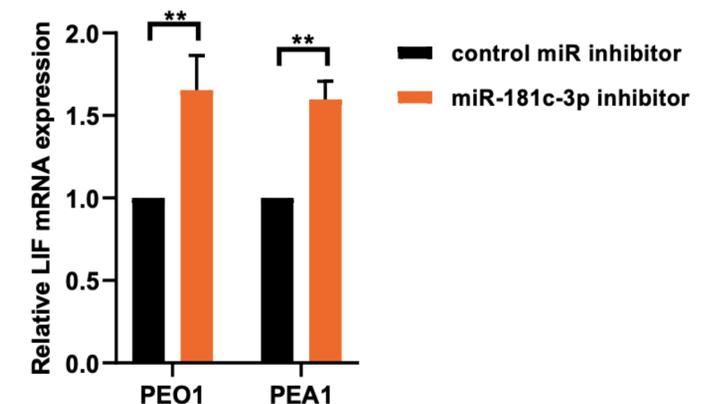


Figure 4: Bar graph representing protein expression of LIF on PEO1 cells treated with miR-181c-3p inhibitor vs PEO1 cells treated with a control.

Conclusions

- Staining indicates higher density of miR-181c-3p in LTS than in STS
- qRT-PCR indicates higher level of miR-181c-3p correlated with increased amount of CD8⁺ T cells in the TME.
- Western Blot and qRT-PCR indicate higher LIF mRNA and protein expression when miR-181c-3p is inhibited.

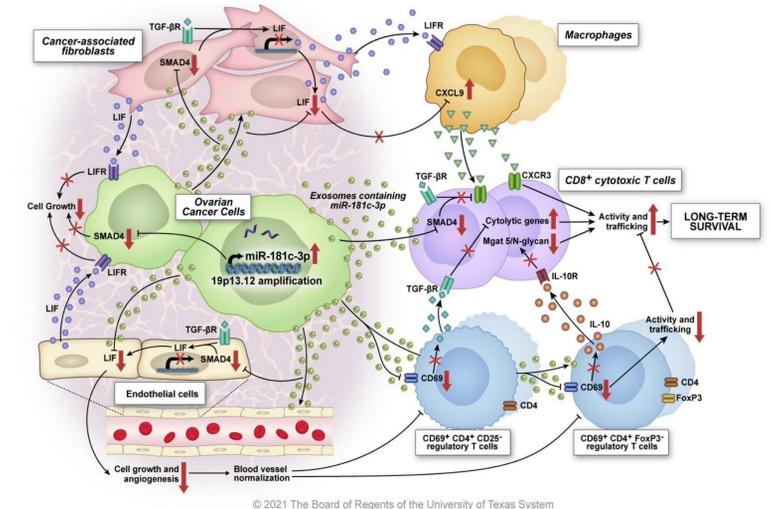


Figure 5: miR-181c-3p down-regulates leukemia inhibitory factor (LIF), which is a pleiotropic cytokine. This allows for decreased binding of LIF to its receptors on macrophages, increasing CXCL9 expression. This then leads to more binding of CXCL9 to CXCR3 on CD8⁺ cytotoxic T cells (CTLs), which activates these CTLs.

References

- 1) Segel, R.L., Miller, K.D., Fuchs, H.E., and Jemal, A., Cancer statistics, 2021. CA Cancer J Clin, 2021. 71(1): p. 7-33.