Biological Function of Nectin 4 in Ovarian Cancer

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Introduction

Ovarian Cancer Overview

- 5th leading cause of cancer death for women in the U.S.
- Most patients are diagnosed after metastasis, when survival rates are low
- Early diagnosis yields 93% five-year survival rate

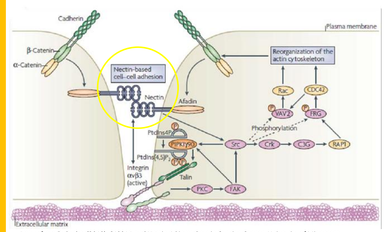


Image from DeRycke, (2010). S100A1 and Nectin 4 Biomarkers in Ovarian Cancer. University of Minnesota Dissertation.

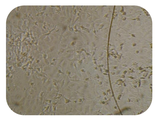
Biomarkers and Nectin 4

- Biomarkers have different properties: early detection, clues to therapy effectiveness, and possible prognostic applications.
- Nectin 4 has been identified by the Skubitz lab as a possible biomarker for ovarian cancer. Nectins belong to a family of immunoglobulin-like proteins important in cell to cell adhesion.
- A study of 500 ovarian cancer tumors showed 48.6% stained for Nectin 4 while 53% of ovarian cancer blood samples tested positive. Nectin 4 also expressed in lung and breast cancer.

Our hypothesis is that knocking down the expression of Nectin 4 will inhibit ovarian cancer cell function and metastasis.

Materials and Methods

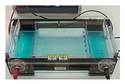
The human ovarian cancer cell line NIH: OVCAR5 was used in these experiments.

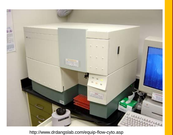


Transfection- The cells were transfected with plasmids containing shRNA constructs TRCN0000061623(23) and TRCN0000061626(26) that targeted the Nectin 4 gene. shRNA's were purchased from the University of Minnesota Biomedical Genomics Center.

Testing for Knockdown:

1. RT-PCR was used to determine RNA levels of cells transfected with shRNA controls parental cells with known expression, and empty vector.
2. Flow cytometry was used to determine Nectin 4 protein levels on the surface of cells based on fluorescence when marked with an antibody.
3. MTT assays determined the metabolic proliferation of the cells.





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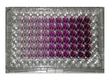

Results

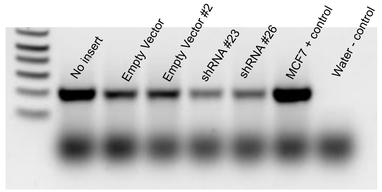

Figure 1. RT-PCR: The two shRNA inserts show definitive knockdown of the expression of Nectin 4 as compared to the Empty Vector samples.

- No insert NIH:OVCAR5 cell RNA without any plasmid added. Two different Empty Vector clones were used to compare to knockdowns. Empty vector cells are NIH:OVCAR5 with plasmid but no shRNA insert.
- shRNA samples include NIH:OVCAR5 + plasmid with the sequence targeting Nectin 4 present.
- MCF7 serve as positive control because of known Nectin 4 expression levels.
- Negative control has no RNA added to sample.

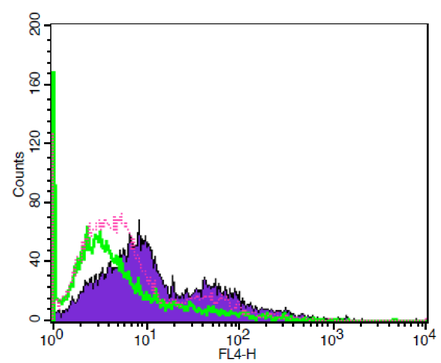

Figure 2. Flow Cytometry: Both of the shRNA inserts display knocked down overall expression of the Nectin 4 protein. shRNA #23 had 75.60% knockdown and #26 had 62.40% knockdown.

- Violet= Empty Vector, Pink = shRNA #26, Green = shRNA #23
- The x-axis shows the relative fluorescence levels of each sample while the y-axis displays the number of cells. Higher levels of fluorescence suggest more Nectin 4 expression.

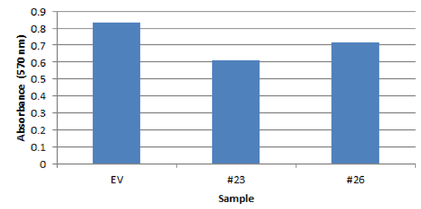

Figure 3. MTT Proliferation Assay: Cells with knocked down Nectin 4 expression with both shRNA inserts show decreased metabolic activity compared to the empty vector.

- Assay is used to determine if Nectin 4 levels affect cell growth.
- Higher absorbance indicates higher metabolic activity in the cells of interest which translates into cell growth.

Summary and Future Directions

Summary

- Both shRNA's lead to knockdown in the expression of Nectin 4 RNA in ovarian cancer cells.
- Lower levels of Nectin 4 RNA in the cancer cells corresponds to a decrease in the Nectin 4 surface proteins.
- Presence of shRNA's promotes change in cell growth rates.
- Results suggest that Nectin 4 levels may affect cell proliferation.

Future Directions

- More analysis on both shRNA's #23 and #26 is needed in order to validate the consequence of knockdown of Nectin 4.
- Test additional shRNA's in similar experiments.
- Perform additional biological assays: scratch assay, to investigate the effect of Nectin 4 on cell movement, a spheroid assay to determine the rate at which cancer cells will form clusters based on the levels of Nectin 4 expression.
- Identifying other biomarkers that interact with Nectin 4.

References

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