The role of microRNA-503 in T-cell deactivation in Colon Cancer

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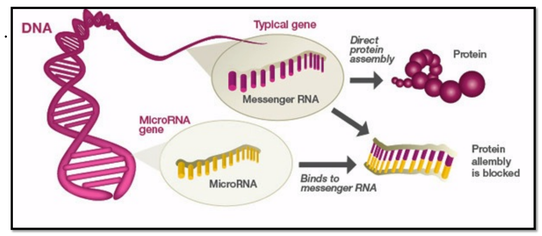
Introduction

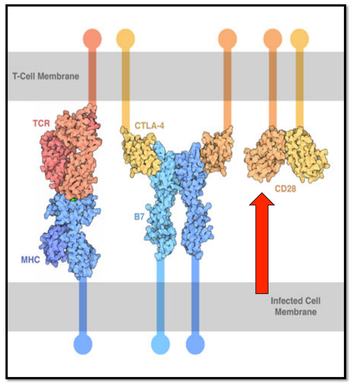
MicroRNA's are short RNA sequences between 22 – 24 nucleotides in length that target complementary messenger-RNA (mRNA) transcripts and prevent them from being translated into proteins. With thousands of different miRNA's in the body, they form an expansive regulatory network which is crucial for proper cell development and function (*1*). Altered microRNA expression is believed to play a role in many diseases, including cancer. In all forms of cancer, miRNA expression levels are noticeably different, and these alterations vary by cancer type. This study focuses on microRNA-503 and its alleged role in intracellular communication between tumorigenic cancer cells and healthy body cells.

Figure 1: Basic overview of post-transcriptional microRNA regulation (*1*)



A common characteristic shared by many cancer types is a lack of immune response in regions of tumor growth due to the immune cells' inability to recognize or target cancer cells. It is widely believed that tumor cells send signals to the body immune cells to retard their overall function and promote uncontrolled tumor proliferation. CD28 (shown below) is a signaling protein found on the extracellular membrane of T-cells, and is involved in T-cell activation and proliferation (*2*). CD28 levels have been found to be reduced in T-cells that are present in the tumor microenvironment, likely due to signaling that occurs between the T-cells and cancer cells. MicroRNA-503 matches a complementary sequence on the CD28 mRNA transcript, and is significantly upregulated in Colon Cancer (*3*).

Figure 2: CD28 T-cell receptor protein (2)



The purpose of this study was to confirm that miR-503 targets the CD28 protein and reduces its expression. Conformation of this regulatory relationship would open up the possibility that miR-503 is involved in the signaling pathway that causes T-cell deactivation in the tumor microenvironment.

Methods

Several experiments were done in an effort to generate data indicating CD28 targeting by miR-503. The final experiment that gave strong evidence for CD28 targeting is outlined below:

- Jurkat T-cells were used as an experimental model, as they are human leukemia cells and thus have lowered CD28 levels. Using anti-microRNA's we reduced the levels of microRNA-503 in the T-cells to observe the resulting effect on CD28 levels.

- Experiment Overview:

Obtained Jurkat T-cells, MZ plasmid vector, and anti-microRNA's: miRzip-182 and miRzip-503

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Insert Anti-miR genes into MZ vector, confirm by restriction digest. Clone vectors containing inserts with PCR

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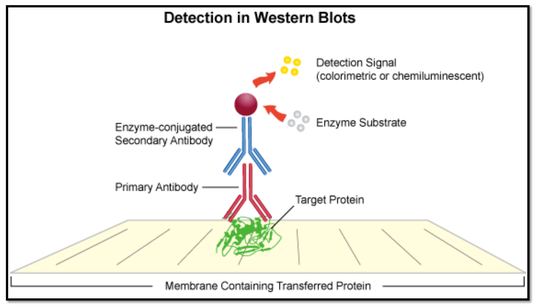
Transfect Jurkat T-cells with MZ vectors containing anti-miRNA's and empty MZ control vector

↓

Extract Jurkat T-cell proteins and determine protein levels with BCA Assay. Run Western Blot with anti-CD28 antibody using beta actin control. CD28 Approximately 46 daltons.

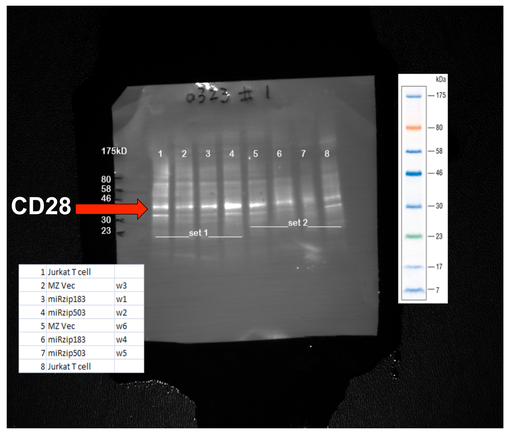
Figure 3: General Overview of Western Blotting Protein Detection Technique (4)



*Additional experiments were done in analyzing miR-503 involvement in T-cell deactivation in the presence of tumors.

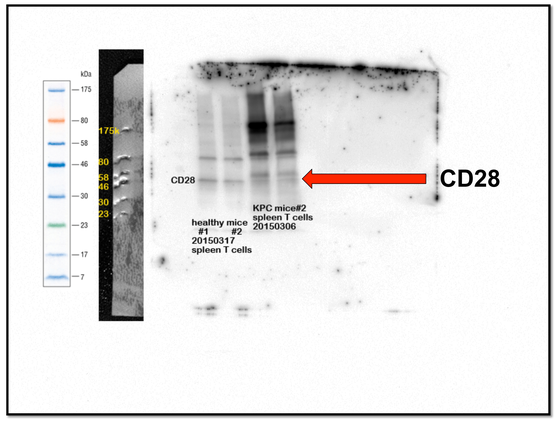
Results

Figure 4: Final Western Blot of Jurkat T-cell transfected with anti-miRNA's, CD28 at 46 kDa.



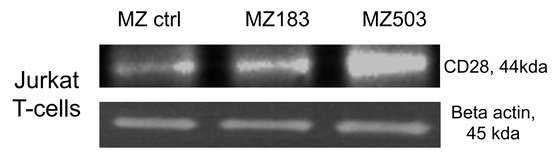
- Lane 1 shows untreated Jurkat T-cells
- Lane 4 shows miRzip-503, high intensity of the band at 46 kDa indicates higher levels of CD28.
- Second set of Westerns had low protein levels as indicated by the faintness of the CD28 bands.

Figure 5: Western Blot comparing CD28 levels between healthy mouse T-cells and T-Cells co-cultured with Colon Cancer cells.



- Lanes 1 & 2 show healthy T-cell CD28 expression levels at 46 kDa.
- Lanes 3 & 4 show CD28 levels of T-cells col-cultured with Colon Cancer cells.
- Faint bands in Lanes 3 & 4 indicate reduced CD28 levels.

Conclusions



While additional experimentation must be done to confirm CD28 as a target of miR-503, the western blot data we obtained strongly supports this conclusion. The image above is a close up of the CD28 bands in Figure 4 for the Jurkat T-cell transfected with the empty MZ vector and MZ vectors containing anti-microRNA's targeting miR-503 and miR-183, also believed to target CD28. The intensity difference in the bands between the untreated Jurkat T-cells and those with miR-503 inhibition is obvious; the restored CD28 levels serve a strong indication that miR-503 targets CD28.

The western blot data in Figure 5 was a follow-up experiment to provide evidence that reduction of CD28 levels in T-cells is caused by cancer cells. Lanes 3 and 4 represent mouse T-cells that were cultured with colon cancer cells and show reduced CD28 levels compared to the healthy T-cells. This data indicates that colon cancer cells manipulate CD28 levels of healthy T-cells while grown in culture with them. In order to connect the dots between the outcomes of these two experiments it is essential to determine the signaling mechanism(s) cancer uses to alter the CD28 levels in T-cells.

A common hypothesis that has been both supported and rejected by researchers is **intercellular exosomal delivery** of genetic materials between cancer cells and body cells, such as microRNA's and other regulatory elements (5). This is currently a focal point of Dr. Li's study in how cancer cells are able to alter the function of T-cells in the tumor microenvironment. Cancer is a highly complex disease and while many of it mechanisms are poorly understood, they all essentially share the common characteristic of promoting tumor proliferation and metastasis. With reduced CD28 levels, T-cells are less capable of fulfilling their adaptive immune function, and this may very well be one of cancer's many mechanisms for promoting its own growth and survival. If so, it is highly probable that microRNA-503 is involved in the process to some extent. However, more experimentation is necessary to confirm either of these hypotheses.

Bibliography

1. Longevinex, **MicroRNAs, Aging and Resveratrol** | Longevinex (2010) (available at *http://www.longevinex.com/articles/micrornas-aging-and-resveratrol/*).

Life Technologies, **T-Cell Receptor (TCR) Overview,** (2015) (available at *http://www.lifetechnologies.com/us/en/home/life-science/cell-analysis/signaling-pathways/t-cell-receptor-tcr/t-cell-receptor-tcr-overview.html*).

University of Minnesota Medical School: Surgery Department – Division of Basic & Translational Research, Subramanian Lab, **Colon Cancer - microRNA Expression Database CC-MED (2014)** *http://www.oncomir.umn.edu/colon/basic_search_result.php?exp1%5B%5D=hsa-miR-503&submit_button=submit*).

1. L. Technologies, **General Western Blot Protocol** - Leinco Technologies (2015) (available at *http://www.leinco.com/general_wb*).

J. A. Tickner, A. J. Urquhart, S.-A. Stephenson, D. J. Richard, K. J. O'Byrne, **Functions and therapeutic roles of exosomes in cancer**., Front. Oncol. 4, 127 (2014).