“Screening for Small Molecules: Target Discovery Against miRNAs for Breast Cancer Treatment”

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Abstract

Breast cancer is one of the leading causes of cancer related deaths and morbidity among women worldwide. Identifying new targets and developing new therapies is very important in order to improve patient outcomes and minimize this health burden. MicroRNAs, a type of non-coding RNA, are potential targets since they can regulate protein expression in a variety of biological and pathological processes, including cancer. We were interested in identifying drugs that could target microRNAs and reverse or arrest the cancer phenotype. To this end, we performed a screening for small molecule inhibitors that can target two oncogenic microRNAs: miR-10b and miR-21. Two cell based models (MDA-MB-231 and MCF07) were chosen to test the ability of these compounds to inhibit these microRNAs. Cells were treated with different concentrations of the compounds at time points. Real-time PCR was performed to determine if the oncogenic microRNAs were effectively inhibited. The results of the screen seem to be dependent on cellular model. Further experiments are required to elucidate the effectiveness of these drugs and organic compounds as well as their mechanism of action.

Introduction

MiRNAs are small non-coding RNAs of approximately 18-24 nucleotides in length which regulate gene expression. These RNAs are produced through the transcription of miRNA genes by RNA polymerase II or III forming a hairpin looped structure known as primary RNA (pri-miRNA). Pri-miRNA is processed into a shorter precursor miRNA (pre-miRNA) by a protein complex consisting of an RNase II enzyme and DiGeorge Critical Region 8. This miRNA exits through Exportin-5, a nuclear membrane protein. Dicer, an RNase II enzyme in the cytoplasm, processes further the miRNA duplex. After unwinding the strands, miRNA can cause mRNA degradation of target mRNA (**Figure 1**).

Breast cancer one of the most common types of cancer worldwide. This disease can be aggressive and invade and metastasize to other tissues. Recent advancements have led to the potential development of small molecule inhibitors against miRNAs in breast cancer that regulate the expression of oncogenes and metastatic progression. Calin and collegues detail the importance of using small molecules for targeting miRNAs, whose expression contributes significantly to the development of cancer.

In this research, we screened for small molecules against overexpressed miRNAs relevant in breast cancer progression. Our hypothesis was that small molecules could specifically target and inhibit overexpressed miRNAs, miR-21 and miR-10b. By inhibiting these miRNAs we would cause cell death in these cancer cells. The small molecules used in this research project are derived from *in silico* predictions by virtual high throughput screening and inhibitor screening libraries. The ultimate goal of this project is to develop novel targeted drug therapies to effectively treat this disease.

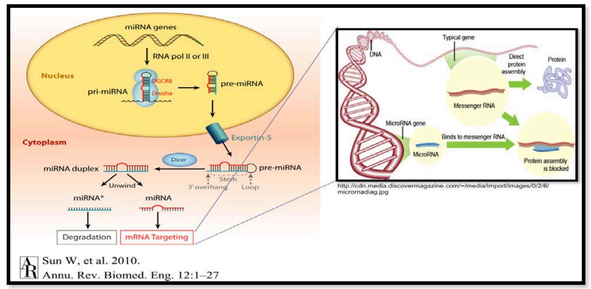
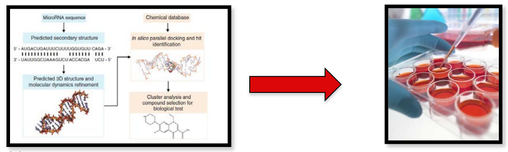


Figure 1. *Processing and general mechanism of action of miRNAs.*

Hypothesis

Small molecules can target and inhibit overexpressed oncogenic miRNAs, miR-10b and miR-21, in breast cancer.

Methods



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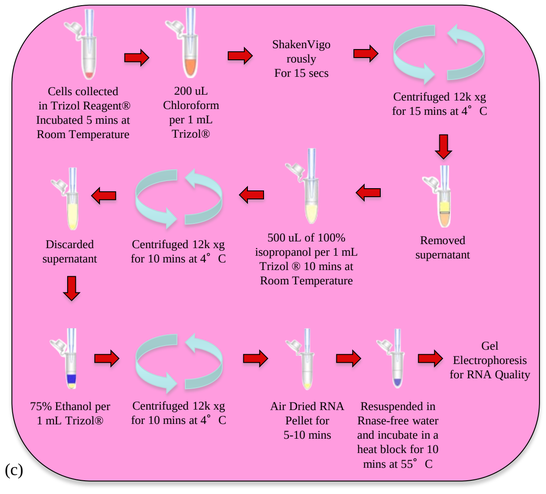

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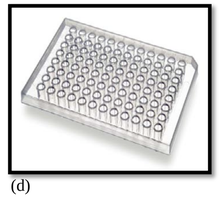
Methods



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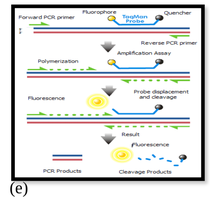


(e)

Figure 2. *Screening for small molecules by evaluation of expressed oncogenic miRNAs after cell treatment.* (a) *In silico* identification by constructing 3D structure models of overexpressed miRNAs in breast cancer and identification of potential hits using newly developed bioinformatics and cheminformatics integrated high throughput docking method to screen databases including 10 million compounds. (b) Small molecule administration in breast cancer cell lines MCF-7 and MDA-MB-231. (c) Detailed diagram of rRNA extraction of samples. (d) Extracted RNA was diluted in Rnase-free water. A master mix was prepared using Reverse Transcriptase Buffer, dNTPs, Rnase Inhibitor, Reverse Transcriptase, and primers. A total volume of 7.5 uL that contained the diluted RNA and master mix was prepared for cDNA preparation. (d) The cDNA samples were used in the Real Time-Polymerase Chain Reaction (RT-PCR) in order to amplify the region of oncogenic miRNAs, miR-10b and miR-21, in these two breast cancer cell lines.

Results

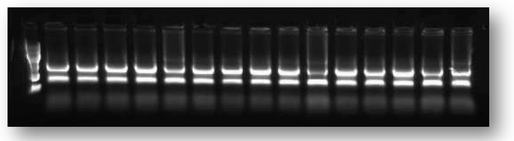
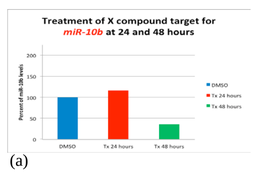
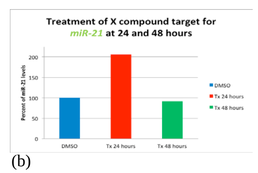


Figure 3. *Gel Electrophoresis of RNA Samples obtained from RNA Extraction.* In this image, it is shown the extracted rRNA of MCF-7 cells after SMI A. Two bands are shown: 28S and 18S. This band pattern was observed in all RNA extractions for verification of RNA quality.



(a)



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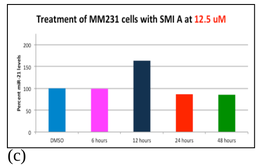
Results



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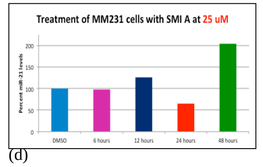

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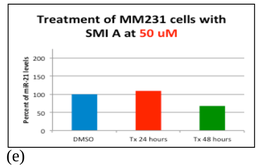

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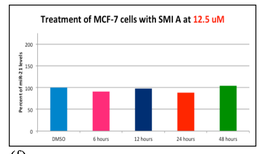

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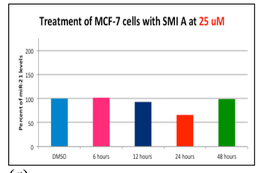

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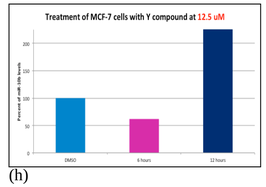

(h)

Figure 4. *Small molecule inhibitors and organic compounds were used to determine the level of expression of miRNAs, miR-10b and miR-21, in breast cancer cells.* In **(a)** and **(b)**, the compound X targets for miR-10b and miR-21 was more effective 48 hours than 24 hours. Treatment of MDA-MD-MM231 breast cancer cells was performed at different time points and concentrations as shown in **(c)**, **(d)**, and **(e).** The percent of miR-21 levels vary in each time point, 6, 12, 24 and 48 hours at the three concentrations studied, 12.5 uM, 25 uM, and 50 uM. In **(f)** and **(g),** the percent of miR-21 levels was decreased at 24 hours when compared to the control of DMSO in both concentrations 12.5 uM and 25 uM. Compound Y was administered in MCF-7 cells and it was highly effective in 6 hours rather than 12 hours as shown in **(h)**.

Conclusions

Further research studies are required to evaluate and elucidate the mechanism of action of these drugs that were designed via *in silico* and inhibitor screening libraries. It is unknown at which level does the drug or compound act, or why it is functional or not against a specific miRNA in the studied breast cancer cell lines. As well, it is relevant to investigate if these small molecule inhibitors and organic compounds selectively affect breast cancer cells, and not normal mammary tissue.

References

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[4] Tili, E., Michaille, JJ, Ghandi, V., Plunkett, W., Smapth, D., and Calin, GA. 2007. miRNAs and their potential for use against cancer and other diseases. Future Oncology. 3:521- 537.

Figure 1b: taken from http://media.theweek.com/img/dir_0068/34444_article_main/scientists-pipette-fluid-into-stem-cell-cultures-researchers-have-discovered-that-cardiac-stem.jpg?175
Figure 1c: microtube diagrams taken from http://eng.bioneer.com/images/products/AccuZol_Total_RNA_fig2.gif
Figure 1e: taken from http://upload.wikimedia.org/wikipedia/en/thumb/0/07/Taqman.png/200px-Taqman.png

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