**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.

**Methods**

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

**Results**

Further research studies are required to evaluate and elucidate the mechanism of action 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 miRNA (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 the cytoplasm, processes further the miRNA duplex. After unwinding the strands, miRNA can cause mRNA degradation of target mRNA (Figure 1).

Breast cancer is one of the most common types of cancer worldwide. This disease can be invasive and metastatic 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 colleagues 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 silico prediction 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.

**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 RNA 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 volume 7.5 uL of RNA that was diluted with master RNA mix prepared DNA synthesis cell lines. The (e) 3D RNA samples were used in the Real-Time Polymerase Chain Reaction (RT-PCR) in order to amplify the region of interest in miRNAs, miR-10b and miR-21, in these two breast cancer cell lines.

**Figure 3**. Gel Electrophoresis of RNA Samples obtained from RNA Extraction. (a) Overexpressed miRNA levels. Cells cultured in Trizol Reagent® at room temperature for 10-15 mins and incubated for 5 mins at 4°C. (b) Centrifuged 12k xg for 10 mins at 4°C. (c) Supernatant discarded. (d) 200 uL Trizol® added for 10 mins at room temperature. (e) Resuspended in 1 uL of Rnase-free water and incubated in a thermocycler at 42°C for 2-5 mins. (f) 2 uL RNA added to 10 uL of Reverse Transcriptase Buffer. (g) Incubated 5 mins at 55°C. (h) Resuspended in 20 uL of Reverse Transcriptase Buffer. (i) Incubated 1 hour at 42°C. (j) Run on 2% agarose gel for 2 hours. Two bands are shown: 28S and 18S. This band quality.

**Figure 4**. Small molecule inhibitors and organic compounds were used to determine the level of expression of miR-21, miR-10b and miR-21 in breast cancer cells. (a) and (b), the compound X targets for miR-10b and miR-21 was more effective 48 hours than 24 hours. Treatment of MDA-MB-231 breast cancer cell lines was performed at different time points and concentrations as shown in (d). (e) and (f). 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 more than 12 hours as shown in (b).

**Conclusions**

Further research studies are required to evaluate and elucidate the mechanism of action of these drugs that were designed via 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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