Datasets exploring putative lncRNA-miRNA-mRNA axes in breast cancer cell lines

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ABSTRACT

Long non-coding RNA (lncRNA)/microRNA (miRNA)/messenger RNA (mRNA) interactions regulate oncogenesis and tumour suppression in breast cancer. Oncogenic lncRNA/miRNA/mRNA axes may offer novel therapeutic targets; therefore, identifying such axes is a clinically relevant undertaking. To explore miRNAs regulated by oncogenic lncRNAs, we queried the NCBI Gene Expression Omnibus (GEO) database to find datasets that profiled gene expression changes upon lncRNA knockdown in breast cancer. We identified four microarray datasets that permitted our interrogation of genes regulated by lncRNAs LincK, LincIN, SPRY4-IT1 and AC009283.1. We specifically analysed changes in miRNA transcripts within these datasets to study miRNAs regulated by each of the four lncRNAs. We subsequently identified the predicted mRNA targets for these miRNAs to uncover possible lncRNA/miRNA/mRNAs axes in breast cancer. These axes may be candidates for future investigation of gene regulation in breast cancer.

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Specifications Table

| Subject | Cancer research |
|---------|----------------|
| Specific subject area | Long non-coding RNA and microRNA interactions in breast cancer |
| Type of data | Figure |
| How data were acquired | The gene expression profiling by array datasets were collected from NCBI's Gene Expression Omnibus (GEO) database (GSE79214, GSE62507, GSE134254, GSE109007). R packages bioMaRt and hoardeR were used to identify lncRNA/miRNA/mRNA axes from these datasets. |
| Data format | Analyzed raw .CEL files from previously conducted array profiling studies. |
| Parameters for data collection | Gene expression profiling by array studies in which a single long non-coding RNA was knocked down in a breast cancer cell line model were considered. |
| Description of data collection | GSE79214: Transcriptome analysis was performed using the GeneChip® Human Gene 2.0 ST Array. GSE62507: Agilent-026652 Whole Human Genome Microarray 4 × 44K v2 (Probe Name version) GSE134254: The Affymetrix Human Transcriptome Array 2.0 [transcript (gene) version] GSE109007: Transcriptome analysis was performed using the GeneChip® Human Gene 2.0 ST Array platform |
| Data source location | GSE79214: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE79214 GSE62507: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62507 GSE134254: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE134254 GSE109007: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE109007 |
| Data accessibility | Repository name: Mendeley Data Data identification number: 10.17632/wstfmr4z57.1 Direct URL to data: https://data.mendeley.com/datasets/wstfmr4z57/draft?access=0e63c44-a3a2-4486-9050-c0dc3b68a3aa Related research article | Venkatesh J, Wasson MD, Brown JM, Fernando W, Marcato P. LncRNA-miRNA axes in breast cancer: Novel points of interaction for strategic attack. Cancer Lett. 2021 Jul 1;509:81-88. doi:10.1016/j.canlet.2021.04.002. |

Value of the Data

- This data is of value for cancer researchers studying lncRNA/miRNA/mRNA interactions in breast cancer. These axes may represent novel therapeutic targets.
- This data may be used to identify specific miRNAs regulated by lncRNAs LincIN, SPRY4-IT1, AC009283.1 and LincK in the associated breast cancer cell lines and their downstream mRNA targets. This may inform lncRNA function.
- These datasets provide the predicted mRNA targets for the majority of miRNAs in the Affymetrix Human Gene 2.0 ST Array [transcript (gene) version], Agilent-026652 Whole Human Genome Microarray 4 × 44k v2 (Probe Name Version) and Affymetrix Human Transcriptome Array 2.0 [transcript (gene) version] arrays.
- Our study provides a foundation for similar analyses using primary or secondary microarray datasets. Our methodology for predicting lncRNA/miRNA/mRNA axes can be applied to explore the regulatory roles of additional lncRNAs of interest.

1. Data Description

Long non-coding RNAs (lncRNAs), microRNAs (miRNAs) and messenger RNAs (mRNAs) form specific interaction networks to regulate gene expression in cancer [1,2]. Here, we have
conducted a network analysis to identify potential lncRNA/miRNA/mRNA axes in breast cancer for oncogenic lncRNAs LincK, LincIN, SPRY4-IT1 and AC009283.1. We provide data illustrating changes in miRNA expression following knockdown of each of the four lncRNAs in breast cancer cell lines to identify potential lncRNA/miRNA interactions. We then predicted the mRNA targets of the lncRNA-regulated miRNAs. Together, these two sets of results reveal potential lncRNA/miRNA/mRNA axes in breast cancer. These data offer a more complete look into the regulatory functions of lncRNAs LincK, LincIN, SPRY4-IT1 and AC009283.1 and may inform the identification of novel targetable axes.

Datasets 1 – 4 contain the probe IDs, HGNC gene symbol, log2 fold change and p-value for each miRNA identified in the primary microarray. They also contain predicted mRNA targets for miRNAs in the TargetScan database [3].

**Dataset 1A. Potential LincIN/miRNA/mRNA axes in MDA-MB-231 breast cancer cells.** The log2 fold change in expression of 1963 miRNAs following LincIN knockdown by shRNA #2 in MDA-MB-231 cells (GSE79214 [4]). This dataset can be used to predict mRNA targets for miRNAs in the Affymetrix Human Gene 2.0 ST Array [transcript (gene) version].

**Dataset 1B. Potential LincIN/miRNA/mRNA axes in MDA-MB-231 breast cancer cells (miRNAs that meet threshold criteria).** The log2 fold change in expression of 225 miRNAs following LincIN knockdown by shRNA #2 that meet the following criteria: log2 fold expression change ≥0.5 or ≤-0.5 and p-value < 0.05. The top hundred LincIN/miRNA/mRNA axes, predicted in silico, are depicted in Fig. 1.

**Dataset 2A. Potential SPRY4-IT1/miRNA/mRNA axis in MDA-MB-231 breast cancer cells.** The log2 fold change in expression of 213 miRNAs following SPRY4-IT1 knockdown in
MDA-MB-231 cells (GSE62507 [5]). This dataset can be used to predict mRNA targets for miRNAs in the Agilent-026652 Whole Human Genome Microarray 4 × 44K v2 (Probe Name version) array.

Dataset 2B. Potential SPRY4-IT1/miRNA/mRNA axis in MDA-MB-231 breast cancer cells (miRNAs that meet threshold criteria). The $\log_2$ fold change in expression of 2 miRNAs following SPRY4-IT1 knockdown that meet the following criteria: $\log_2$ fold expression change $\geq 0.5$ or $\leq -0.5$ and p-value $< 0.05$. The top twenty SPRY4-IT1/miRNA/mRNA axes, predicted in silico, are depicted in Fig. 2.

Dataset 3A. Potential AC009283.1/miRNA/mRNA axis in SKBR3 breast cancer cells. The $\log_2$ fold change in expression of 1874 miRNAs following SPRY4-IT1 knockdown in SKBR3 cells (GSE134254 [6]). This dataset can be used to predict mRNA targets for miRNAs in the Affymetrix Human Transcriptome Array 2.0 [transcript (gene) version].

Dataset 3B. Potential AC009283.1/miRNA/mRNA axis in SKBR3 breast cancer cells (miRNAs that meet threshold criteria). The $\log_2$ fold change in expression of eight miRNAs following AC009283.1 knockdown that meet the following criteria: $\log_2$ fold expression change $\geq 0.5$ or $\leq -0.5$ and p-value $< 0.05$. The top hundred AC009283.1/miRNA/mRNA axes, predicted in silico, are depicted in Fig. 3.

Dataset 4A. Potential LincK/miRNA/mRNA axis in MCF7 breast cancer cells. The $\log_2$ fold change in expression of 1963 miRNAs following LincK knockdown by shRNA #1 in MCF7 cells (GSE109007 [7]). This dataset can be used to predict mRNA targets for miRNAs in the Affymetrix Human Gene 2.0 ST Array [transcript (gene) version].

Dataset 4B. Potential LincK/miRNA/mRNA axis in MCF7 breast cancer cells (miRNAs that meet threshold criteria). The $\log_2$ fold change in expression of 45 miRNAs following LincK knockdown by shRNA #1 that meet the following criteria: $\log_2$ fold expression change $\geq 0.5$ or $\leq -0.5$ and p-value $< 0.05$. The top hundred LincK/miRNA/mRNA axes, predicted in silico, are depicted in Fig. 4.

2. Experimental Design, Materials and Methods

The data analysis pipeline used to profile putative lncRNA/miRNA/mRNA axes is summarized in Fig. 5. To identify potential lncRNA/miRNA/mRNA axes in breast cancer, we searched for studies that profiled changes in gene expression in the context of lncRNA knockdown in a
Fig. 3. Predicted AC009283.1/miRNA/mRNA axes in SKBR3 breast cancer cells. The ten miRNAs with the highest absolute log₂ fold expression change and p-value < 0.05 upon lncRNA AC009283.1 knockdown by shRNA in SKBR3 cells (obtained from the NCBI GEO GSE134254 dataset) with mRNA targets (predicted via TargetScan) are shown. Up to ten of the strongest mRNA targets for each of these miRNAs (predicted via TargetScan) are plotted. Together, these interactions reveal one hundred potential miRNA/mRNA interactions stemming from AC009283.1 in SKBR3 cells. The miRNAs, their fold expression change induced by SPRY4-IT1 knockdown, and their predicted mRNA targets shown in this plot are detailed in Dataset 3A and Dataset 3B. This graph was generated using the igraph package in Rv4.0.4 [8].

Fig. 4. Predicted LincK/miRNA/mRNA axes in MCF7 breast cancer cells. The ten miRNAs with the highest absolute log₂ fold expression change and p-value < 0.05 upon LincK knockdown by shRNA #1 in MCF7 cells (obtained from the NCBI GEO GSE109007 dataset) with mRNA targets (predicted via TargetScan) are shown. Up to ten of the strongest mRNA targets for each of the miRNAs (predicted via TargetScan) are plotted. Together, these interactions reveal one hundred potential miRNA/mRNA interactions stemming from LincK in MCF-7 cells. The miRNAs, their fold expression change induced by LincK knockdown, and their predicted mRNA targets shown in this plot are detailed in Dataset 4A and Dataset 4B. This graph was generated using the igraph package in Rv4.0.4 [8].
breast cancer cell line on the GEO database. We identified four studies that met these criteria: GSE79214 [4], GSE62507 [5], GSE134254 [6], and GSE109007 [7]. The GSE79214 study used two shRNAs to knockdown LincIN in triple negative breast cancer MDA-MB-231 cells[4]. Our analysis compares the changes in gene expression between the control and shRNA #2-treated samples. The authors of this study performed transcriptome analysis using the GeneChip® Human Gene 2.0 ST Array.

The authors of the GSE62507 study knocked down lncRNA SPRY4-IT1 using short interfering RNAs (siRNAs) in MDA-MB-231 cells[5]. Changes in gene expression levels between the siRNA and control samples were performed using the Agilent-026652 Whole Human Genome Microarray 4 × 44K v2 (Probe Name version).

LncRNA AC009283.1 was knocked down using short hairpin RNAs (shRNAs) in the HER2-negative breast cancer cell line SKBR3 in the GSE134254 study [6]. Changes in gene expression between the treatment and control samples were obtained through the Affymetrix Human Transcriptome Array 2.0 [transcript (gene) version].
The GSE109007 study used two shRNAs to knockdown LincK in ER+ MCF-7 breast cancer cells [7]. Our analysis tracks the changes in gene expression between the control and shRNA #1-treated samples obtained using the GeneChip® Human Gene 2.0 ST Array platform.

The log₂ fold change (log₂FC) expression of the genes between the lncRNA knockdown and control samples were computed using the NCBI GEO2R tool (https://www.ncbi.nlm.nih.gov/geo/geo2r/). The summary data was downloaded and imported into RStudio (running R v4.0.2) [9,10]. Array probe IDs for GSE79214 (AFFY HuGene 2.0 st v1 probe set), GSE10907 (AFFY HuGene 2.0 st v1 probe set), GSE62507 (AGILENT WholeGenome 4×44k v2 probe set) and GSE134254 (AFFY HTA 2.0 probe set) were converted to HNGC gene symbols using the “biomaRt” R package (from the Bioconductor project) [11–13] (the code is provided in file identifying_axes.R).

The transcript type (i.e. protein-coding, miRNA, long non-coding RNA, etc.) for each probe was determined using the “biomaRt” R package (using the “gene_biotype” attribute). Probes with a gene_biotype value of “miRNA” were extracted and any duplicate entries in the datasets were removed. The fold change of the miRNA genes upon lncRNA knockdown were visualised using the “EnhancedVolcano” R package (from the Bioconductor project) [13,14] (the code is provided in file identifying_axes.R).

The predicted mRNA targets for each miRNA were acquired using the “hoardeR” R package, which calls the TargetScan [3] API via the targetScan() function (the code is provided in file identifying_axes.R). This data was merged with the miRNA fold change data to create the Datasets 1A, 2A, 3A and 4A. Each dataset was filtered to only include miRNAs with a log₂ fold expression change ≥0.5 or ≤−0.5 and p-value < 0.05. These miRNAs represent the strongest lncRNA-interacting candidates. This data is located in Datasets 1B, 2B, 3B, 4B.

**Ethics Statement**

NA

**CRediT Author Statement**

**MCW**: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review & Editing; **JMB**: Conceptualization, Writing – Original Draft, Writing – Review & Editing; **JV**: Writing – Review & Editing; **WF**: Writing – Review & Editing; **PM**: Conceptualization, Methodology, Validation, Writing – Review & Editing, Supervision, Project administration, Funding acquisition.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

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