The Role of IncRNA in ER+ Breast Cancer

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Abstract

The role of long non-coding ribonucleic acids (IncRNA) in breast cancer still needs in depth study. IncRNAs have been shown to have functions in transcriptional, post-transcriptional, and epigenetic regulation. Most notably the IncRNA HOTAIR has been shown to interact with ER-alpha, enhancing its signal, and driving progression in tamoxifen-resistant breast cancer. In this project, we seek to analyze available public GRO-seq, RNA-seq, and ChIP-seq datasets to find correlation between IncRNAs and estrogen response in breast cancer cells.

One candidate, namely LINC00160, passed all three relevance tests. Therefore, we can state with the highest relative confidence that LINC00160 is involved in ER-positive breast cancer. LINC00160 expression positively correlates with the expression of the nearby coding gene RUNX1, a gene which produces a transcription factor believed to be involved in a complex of chromatin modifying enzymes, possibly facilitating ERα binding to promoter regions of ERα target genes. According to GRO-seq data, at both of these sites, bidirectional transcription occurs, producing transcripts approximately 1 kilobase (kb) in length. RNA-seq data confirms that transcription also occurs along the entire region of LINC00160. In a previous study, silencing of LINC00160 in MCF-7 cells using shRNA reduced cell proliferation. In the study, however, only RNA-seq data was analyzed in the selection of LINC00160 as a candidate for silencing, so it is unlikely that the enhancer model was taken into consideration in the IncRNA design.

It is possible that the phenotypic response was affected by unintentional silencing of the eRNA transcripts within the gene body of LINC00160.

We believe that the intronic LINC00160 co-exists with two enhancer RNA (eRNA) and that the region is an enhancer of RUNX1.

Figure 1. (A) The intersection of significantly up- and down-regulated IncRNA genes by E2 stimulation from both GRO-seq and RNA-seq was compared with differential binding sites of ERα gathered by ChIP-seq with E2 treatment. (B) The transcripts of IncRNA genes in MCF7 cells regulated by E2.

Figure 2. (A) The top 20% most highly expressed IncRNA genes under E2 stimulation were selected if they had neighboring (< 50kb) coding genes from poly-A selected RNA-seq data with differential expression (LFC > 0.5, p-value < 0.005) and had significant Pearson correlation (correlation coefficient > 0.2, p-value < 0.05) with the coding neighbor in patient samples from TCGA. (B) The lower patient expression levels of RUNX1 and LINC00160 were positively correlated.

Figure 3. The top 20% most highly expressed IncRNA genes under E2 stimulation were analyzed in breast cancer patient samples from The Cancer Genome Atlas (TCGA). The survival rates of the 25% of ER+ patients with the highest expression of each gene were compared with those of the 25% with the lowest expression using Kaplan-Meier survival estimation. Genes with a significant difference in survival (p-value < 0.1) were cross-referenced with response to E2 and proximity to ERα.

Figure 4. (A) Bidirectional transcription within the gene body of LINC00160 and binding site of ERα. (B) Upregulation of LINC00160 and RUNX1 under E2 stimulation. (C) Steady state expression of LINC00160 at exons.

Figure 5. Summary of work flow

Acknowledgements

Grants: NIH/NCI P20 CA165589/UTHSCSA, P20 CA165583/UTSA, SALSI. Dr. Jason Liu, Dr. Zhao Zhang. The study was supported by NIH U54 CA217297 grant as well as Cancer Prevention Institute of Texas’ single cell core fund.