Antisense long non-coding RNAs in breast cancer: A transcriptome-wide disruption

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Why breast cancer?

- Most frequent cancer type in women (~35% of female cancers)
- First cause of cancer death in women (~35% of cancer deaths)
- ⅛ women will have breast cancer during their lifetime

- Breast cancer involves multiple genes
- Genetic alteration mechanisms are not always well known
- Some of these mechanisms involve antisense lncRNAs

Why antisense lncRNAs?

- Long non-coding RNAs (lncRNAs) = non protein-coding transcripts longer than 200 nt
- Antisense lncRNA or natural antisense transcript (NAT) = lncRNA
  - Sharing the same genomic location as a protein-coding gene
  - Transcribed in the opposite direction
  - Overlapping > 1 exon
- NATs
  - Regulate protein-coding gene expression
  - Overlap more than 50% of sense RNA transcripts
  - Have a lower expression than protein-coding genes
  - Can have an effect in *cis* or in *trans*

Study design

23 ER+/HER- breast cancer patients
- Tumors
- Adjacent healthy tissue

DNA → CGH, RT-qPCR, External microarray data → Validation
- RNA-Seq fold-changes vs. CGH (all genes)
- RNA-Seq fold-changes vs. microarray fold-changes (external study)
- RNA-Seq vs. RT-qPCR (subset of genes)
- Principal Components plot (tumors vs. healthy)

RNA → Strand-specific RNA-Seq → Reads alignment, QC, Gene quantification, Normalization

varRatio analysis:

$$tumoral\ read\ counts\ ratio = \frac{\sum tumor\ read\ counts_{antisense}}{\sum tumor\ read\ counts_{protein\ coding}}$$

$$normal\ read\ counts\ ratio = \frac{\sum normal\ read\ counts_{antisense}}{\sum normal\ read\ counts_{protein\ coding}}$$

$$varRatio = \frac{tumoral\ read\ counts\ ratio}{normal\ read\ counts\ ratio}$$

Differential correlation analysis (DGCA)

Differential expression analysis (DESeq2)

extreme values / p-val < 0.05 / p-val < 0.05 → Lists of antisenses / protein-coding pairs

TCGA Data: 1066 RNA-Seq Breast Cancer samples → Survival analysis

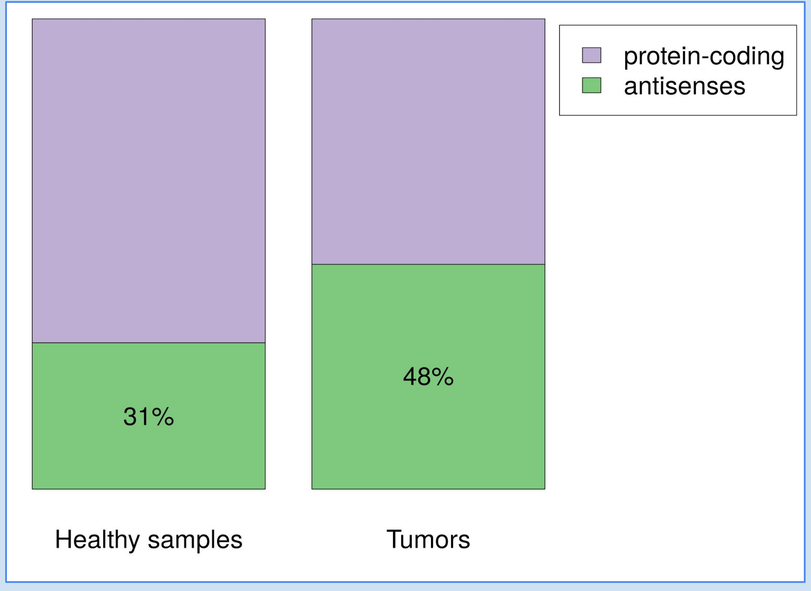
RNA-Seq pipeline | Validation of RNA-Seq data | Antisenses selection methods | Filtering of significant / extreme antisenses | Test for enrichment in survival-associated genes

Goal

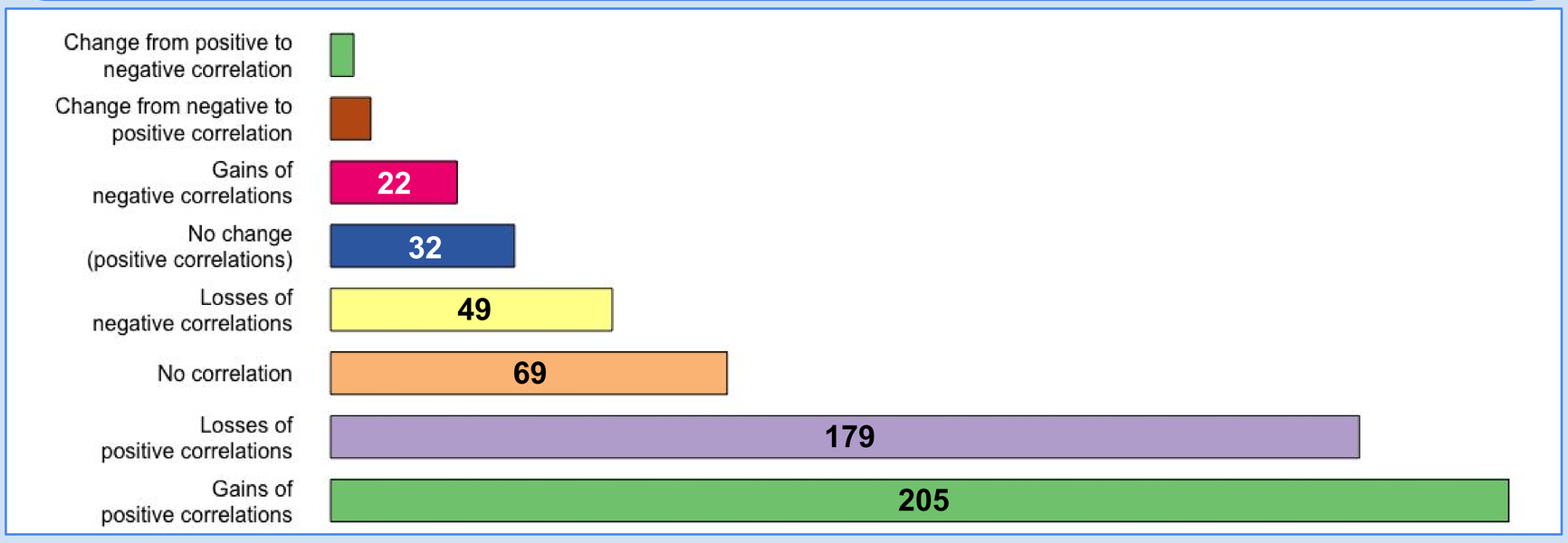
- **Global**: observe the disruption of the antisense lncRNA landscape in breast cancer tumors, at the whole transcriptome scale
- **Local**: use analytical methods to highlight pairs of antisense lncRNAs and overlapping protein-coding genes involved in the breast cancer pathology

Results

1. Global **over-expression** of antisense lncRNAs in breast cancer tumors



2. **Correlations between pairs** of antisense lncRNAs and **overlapping** protein coding genes are disrupted in breast cancer tumors, with **positive correlations** more frequently affected (**3.3** times) than negative ones.



3. Protein coding genes overlapping antisense lncRNAs highlighted by our selection methods are **1.3** times more likely than protein coding genes without antisense overlap to be **associated with survival**.

(survival analysis performed on 1066 TCGA breast cancer samples)

Conclusion

This is the first breast cancer-based, transcriptome-wide, strand-specific RNA-Seq study performed with paired tumor and adjacent tissue samples. Our results show that opposite strand transcription regulation might play a key role in the breast cancer disease, involving several different protein-coding genes and antisenses. Further functional molecular studies will be needed to explore the mechanisms and roles of specific antisenses.

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