Transcriptome wide analysis of natural antisense transcripts shows potential role in breast cancer

Stephane Wenric, Sonia El Guendi, Jean-Hubert Caberg, Warda Bezzaou, Corinne Fasquelle, Benoit Charloteaux, Latifa Karim, Benoit Hennuy, Pierre Frères, Joëlle Collignon, Meriem Boukerroucha, Hélène Schroeder, Fabrice Olivier, Véronique Jossa, Guy Jérusalem, Claire Josse, Vincent Bours

Why breast cancer?

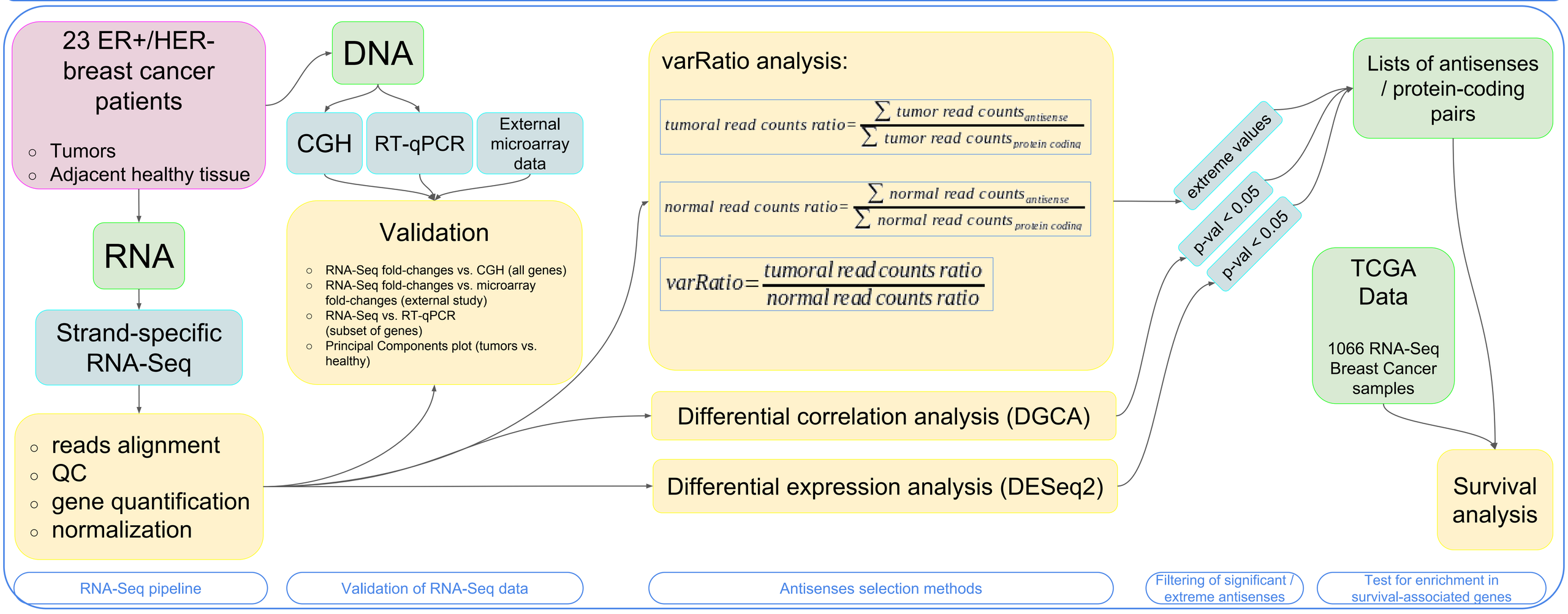
- Most frequent cancer type in women (~35% of female cancers)
- First cause of cancer death in women (~35% of cancer deaths)
- ⅛ women will have breast cancer during their lifetime

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

Why antisense lncRNAs?

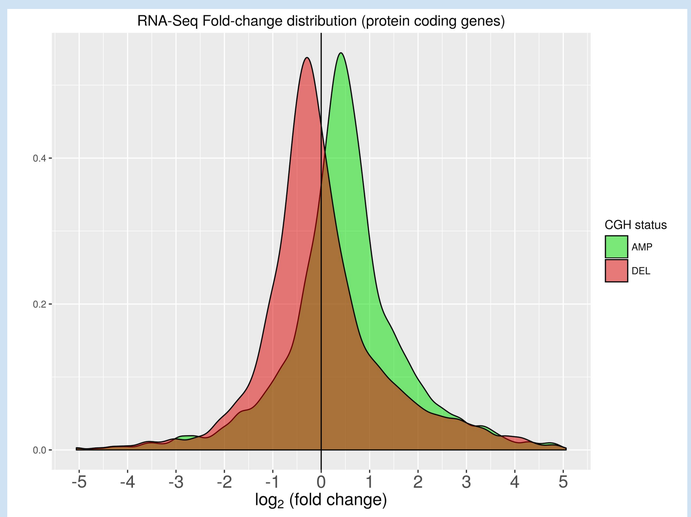
- Long non-coding RNAs (lncRNAs) = non protein-coding transcripts longer than 200 nt
- Antisense lncRNA or natural antisense transcript (NAT) = lncRNA
  - Sharing the same genomic location as a protein-coding gene
  - Transcribed in the opposite direction
  - Overlapping > 1 exon
- NATs
  - Regulate 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

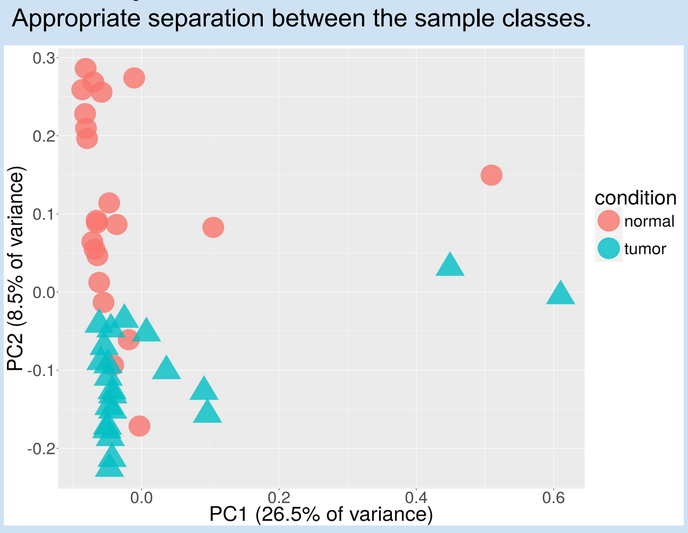


Validation

RNA-Seq vs. CGH:
Overall expression levels of coding gene transcripts inside genomic amplification or deletion newly acquired in the tumor were respectively increased and decreased.



RNA-Seq PCA:
Appropriate separation between the sample classes.

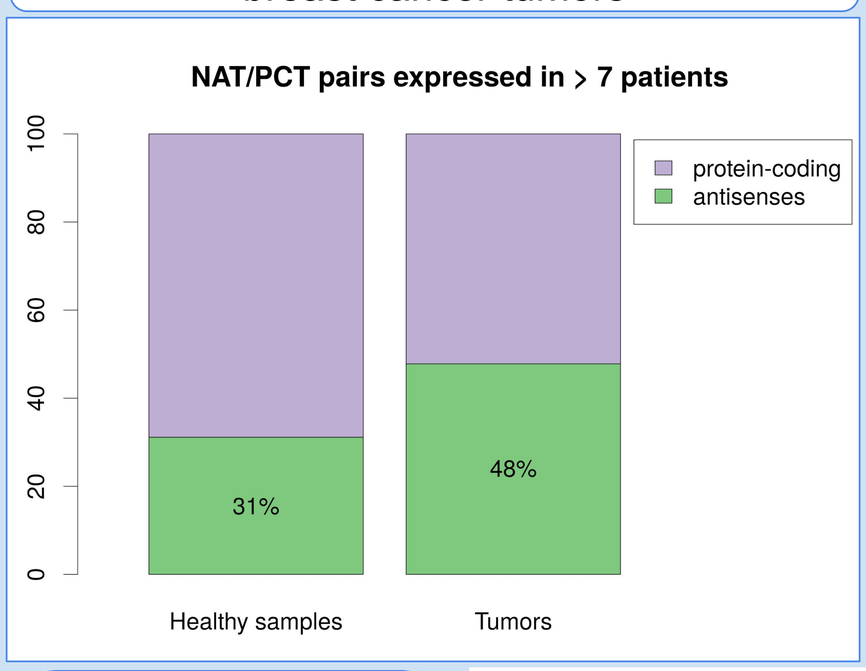


RNA-Seq vs microarray:
Comparison with GEO Dataset GSE65216
- Average Spearman correlation: 0.613 (p-val < 0.001)
- 76.6% of genes modulated in the same direction

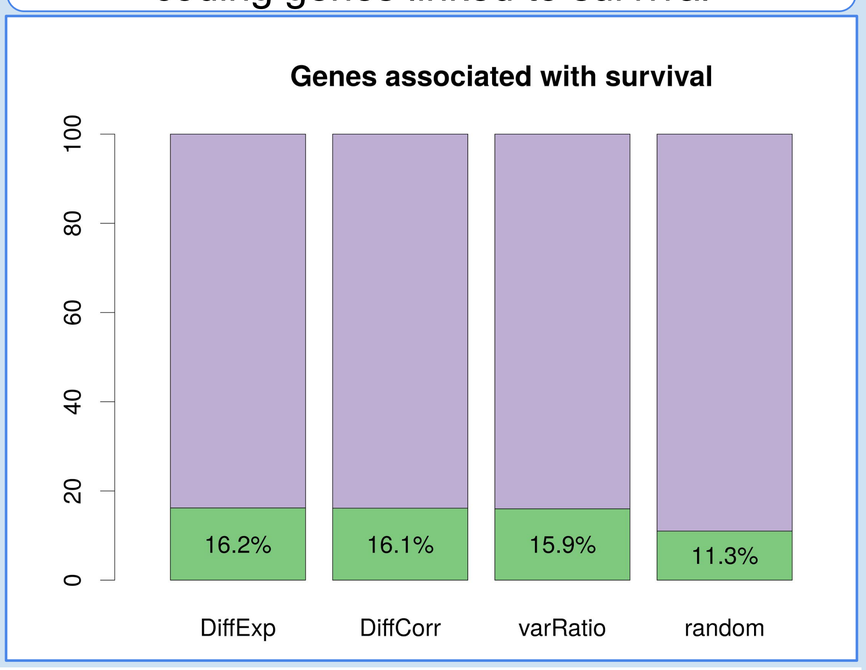
RNA-Seq vs RT-qPCR:
Small set of genes (protein coding and antisenses)

Results

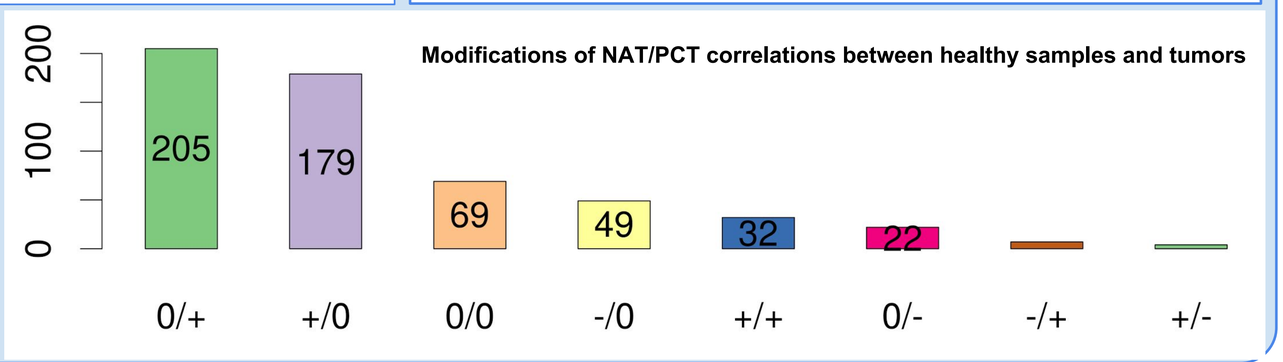
Global over-expression of antisenses in breast cancer tumors



Highlighting of pairs of antisense/protein coding genes linked to survival



Disruption of positive correlations between antisenses and protein-coding genes in breast cancer tumors



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.

Funding: French Community of Belgium, Belgian Funds for Scientific Research (F.R.S.-FNRS), F.R.S.-FNRS-Télévie, CHU Liège (F.I.R.S.), Wallonia (XSPRELTRIN), CÉCI (F.R.S.-FNRS Grant 2.5020.11)