Assessing functional ER pathway activity using a computational pathway model

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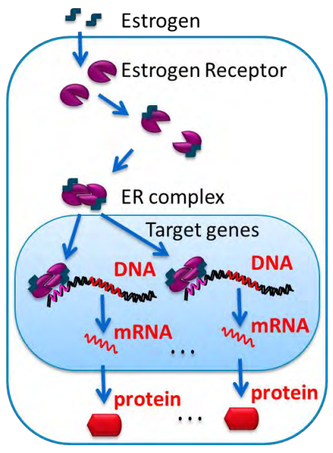
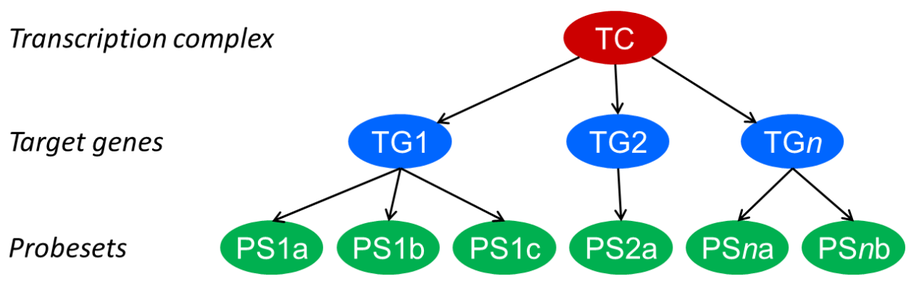
Introduction

In breast cancer, patients with a positive histopathology test for estrogen receptor (ER) are eligible for hormonal treatment, but clinical practice has shown that up to 50% of them do not respond to this treatment. Therefore, we have developed a computational model for assessing functional activity of the ER pathway in an individual patient, which we hypothesize to be more informative of therapy response.



Material and method

We have modeled the transcriptional program of the ER pathway, to infer functional ER pathway activity from mRNA levels of the direct target genes, measured on Affymetrix HG-U133Plus2.0 arrays (fRMA preprocessed) [1]. We modeled this in a probabilistic manner, using a Bayesian network, with three types of nodes: a transcription complex, target genes and probesets. The model describes (i) how the expression of the target genes depends on the activation of the transcription complex, and (ii) how probeset intensities depend in turn on the expression of the respective target genes.



The model can be used to estimate pathway activity in a certain test sample by entering the Affymetrix probeset measurements, and inferring backwards in the model what the probability must have been for the transcription complex to be present.

Target gene selection

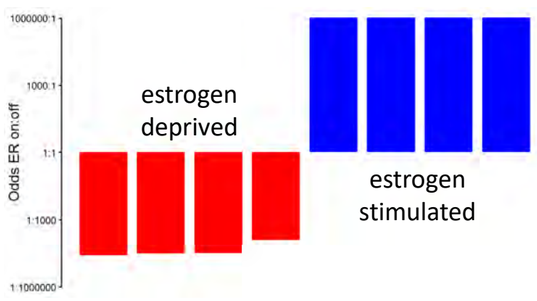
For optimal performance, the models should contain (only) direct target genes of the respective pathways. We selected them through an exhaustive literature search, based on multiple sources of evidence:

- motif analysis of regulatory regions
- transcription factor binding experiments (CHiP-chip/seq/pcr)
- differential expression analysis (activated vs. non-activated pathway)

This resulted in a list of 27 well-validated ER target genes. This number gives specific results on the one hand, and a robust model on the other hand.

Model calibration

The probabilistic relations in the Bayesian models need to be made quantitative, to allow for quantitative probabilistic reasoning. The parameters describing the relation between the transcription complex and target genes have been hand-picked based on earlier experiments. The parameters related to the probeset nodes have been fitted on data from MCF7 cell line experiments with known activity status, by either depriving them from estrogen, or stimulating them with estrogen.



ER pathway results on the calibration dataset (GSE8597)

RT-qPCR based model

Next to a microarray-based model, we also developed a RT-qPCR based model, using a subset of 12 ER target genes, which were chosen based on literature evidence and discriminating power. For each of the 12 target gene we developed an RT-qPCR assay. For normalization, we chose 7 reference genes.

Biological validation

14 breast cancer cell lines were used for biological validation: all cultured in absence of estrogen. After 48 hours, estradiol (E2) was added to the cells in culture according to the schedule below.

ER pathway unstimulated

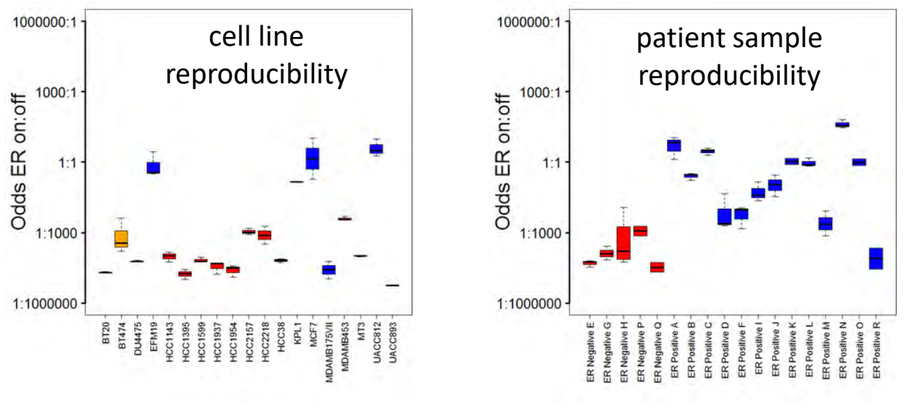
ER pathway stimulated

Conclusions

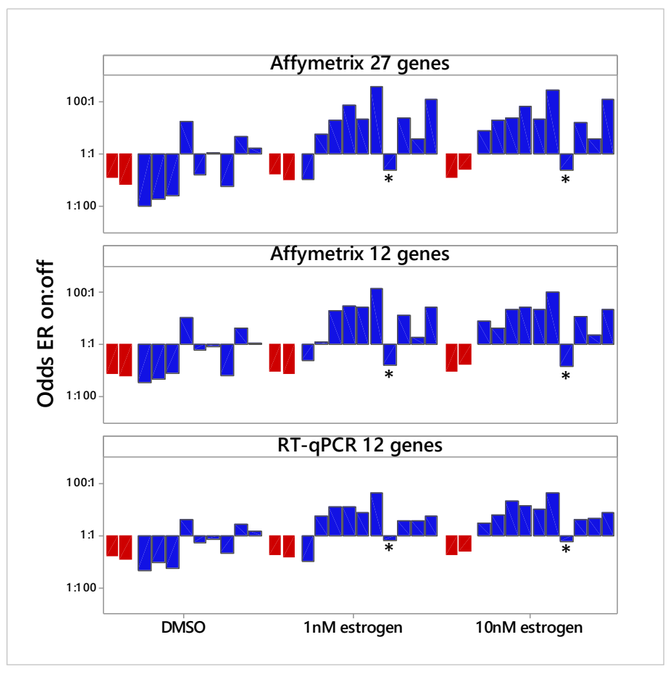
- **We developed a computational model to predict functional ER pathway activity in an individual sample using mRNA expression data.**
- **A positive ER staining is a prerequisite, but not sufficient for ER pathway activity.**
- **ER pathway activity correlates with better prognosis and therapy response.**
- **Clinical validation and other pathways are under study.**

Results

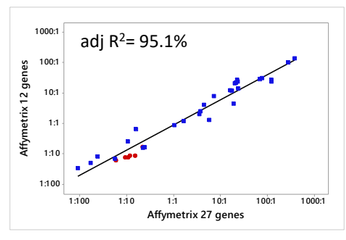
Replication experiments with multiple microarrays and samples show good reproducibility of activity predictions:

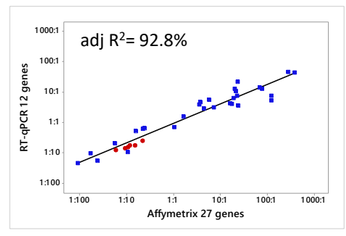


Good agreement between microarray and RT-qPCR based activity predictions:

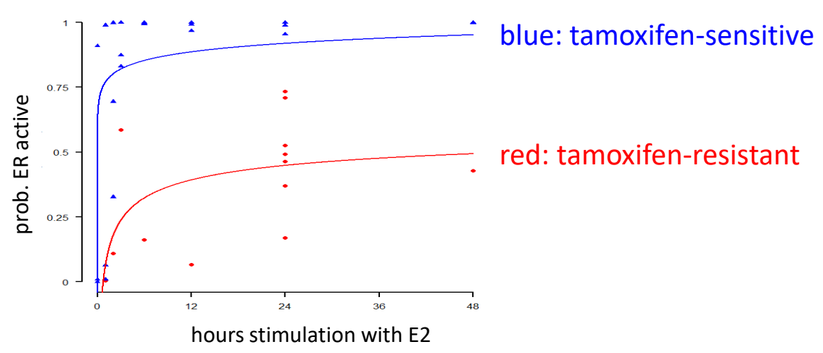


blue: ER positive cell lines
red: ER negative cell lines
(* negative in own ER staining)

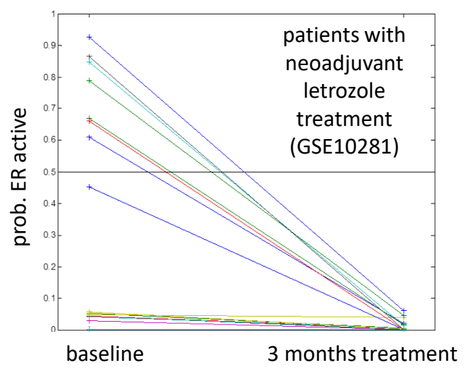


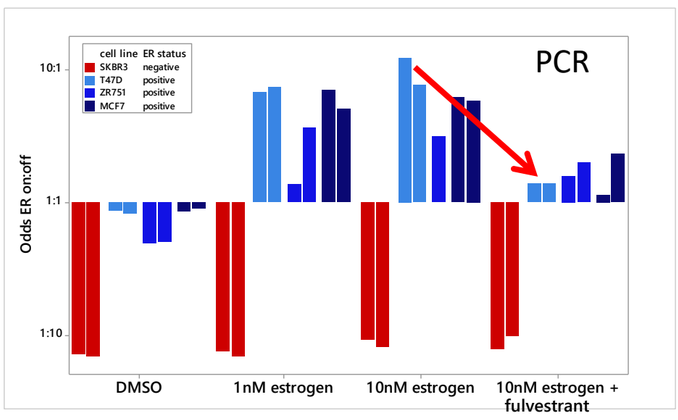


There is a clear difference in ER pathway activity between tamoxifen-sensitive and tamoxifen-resistant MCF7 cell lines upon stimulation with estrogen:

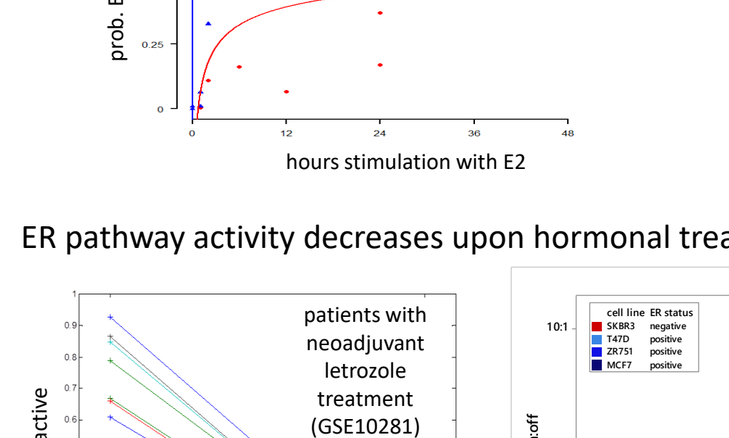


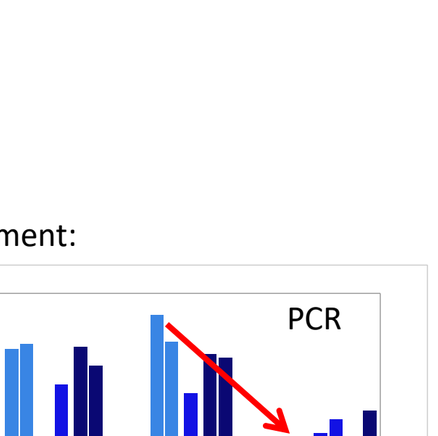
ER pathway activity decreases upon hormonal treatment:





An active ER pathway is observed in practically no ER-negative sample and in 37% of ER-positive samples (GSE12276, GSE21653, GSE9196, GSE6532). Within the ER-positive group, ER activity is correlated with better relapse-free survival.





[1] W. Verhaegh et al. Selection of personalized patient therapy through the use of knowledge-based computational models that identify tumor-driving signal transduction pathways. *Cancer Res*. 2014 Jun 1;74(11):2936-45.

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