Predicting first line tamoxifen response of recurrent ER+ breast cancer patients based on transcriptional activity of signaling pathways

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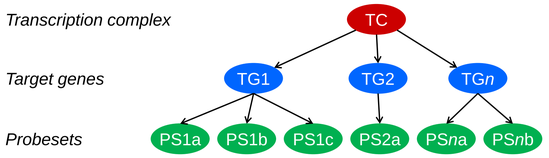
Summary

- **We developed computational models to assess *functional* activity of the ER, Wnt, AR, PI3K, HH, NFκB and TGFβ pathways in individual samples, using mRNA expression data.**
- **We assessed activity of these pathways on 152 ER+ breast cancer patients that received first line tamoxifen treatment**
  - **132 M0 patients with a recurrence (no adjuvant tamoxifen)**
  - **20 M1 patients**
- **Outcome: progression free survival (PFS) and RECIST response**
- **In the 132 M0 patients:**
  - **ER pathway activity is associated with favorable PFS**
  - **TGFβ and AR pathway activity are associated with shorter PFS**
  - **TGFβ pathway activity is associated with worse response**
- **In the 20 M1 patients:**
  - **HH pathway activity is associated with shorter PFS and worse response.**

Material & method

Pathway activities. We have modeled the transcriptional programs of the ER, Wnt, AR, PI3K, HH, NFκB and TGFβ pathways, to infer functional pathway activity from mRNA levels of their direct target genes, measured on Affymetrix HG-U133Plus2.0 and +PM arrays (fRMA preprocessed). Details of the approach are described in [1]. We modeled the pathways in a probabilistic manner, using a Bayesian network, with three types of nodes: a transcription complex, target genes and probesets. Each model describes (i) how the expression of the target genes depends on the activation of the respective transcription complex, and (ii) how probeset intensities depend in turn on the expression of the respective target genes.



The models can be used to estimate pathway activity in an individual test sample by entering its Affymetrix probeset measurements, and inferring backwards in the model what the probability is that the transcription complex must have been present.

Patient samples. Pathway activities were determined on 152 ER+ breast cancer patient samples from Erasmus MC that all received first line tamoxifen treatment of their metastases or recurrence.

Outcome data that was used is progression free survival (PFS) and response according to RECIST criteria. Additional clinical data was also used.

Clinical and biological factors	No. of patients†
Age at primary surgery	
≤ 50 years	62
> 50 years	90
Menopausal status at primary surgery	
pre-menopausal	46
peri-menopausal	7
post-menopausal	80
Tumor grade	
good/moderate	24
poor	82
Tumor size primary tumor	
≤ 2 cm	44
> 2- ≤5 cm	82
> 5 cm + pT4	19
M-stage primary tumor	
M0, no distant metastases present	132
M1, distant metastases present	20
Nodal status primary tumor	
N0, no positive lymph nodes	79
N1+N2, positive lymph nodes	73

Clinical and biological factors	No. of patients†
Age at start 1st line Tamoxifen	
≤ 50 years	45
> 50 years	107
***ERBB2* primary tumor**	
low, < 18	124
high ≥ 18	16
***PGR* primary tumor**	
low, < 6.2	44
high ≥ 6.2	108
Disease free interval	
≤ 1 yr	35
1- 3 yr	60
> 3 yr	57
Adjuvant systemic therapy	
none	128
chemotherapy	24
Dominant site of relapse	
local regional	18
bone	83

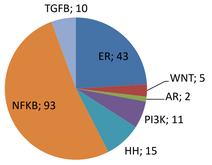
Because of others and unknowns, numbers do not always add up to 152.
Abbreviations: *PGR*, progesterone receptor Affymetrix mRNA level; *ERBB2*, HER2/ERBB2 RTqPCR mRNA level

Statistical analysis. We assessed association of pathway activities to PFS using multivariate Cox proportional hazards regression, separately and in combination with traditional response prediction factors. This was complemented by Kaplan-Meier analysis of PFS, and Anova and Wilcox rank sum tests on response groups.

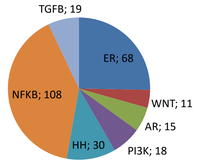
Resulting pathway activities

On the 152 samples, 121 (80%) had at least one pathway active, which is defined as having an inferred probability above 0.5. If we lower the threshold to 0.2 (called marginally active), we get a number of 141 samples (93%). Furthermore, we often see combinations of activity.

# Pathways	Active in # patients (%)	Marginally active in # patients (%)
0	31 (20%)	11 (7%)
≥ 1	121 (80%)	141 (93%)
≥ 2	47 (31%)	86 (57%)
≥ 3	11 (7%)	32 (21%)



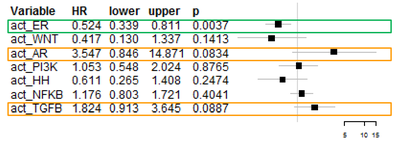
Active pathways (P > 0.5) in samples



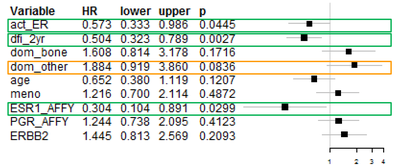
Marginally active pathways (P > 0.2) in samples

132 M0 patients with recurrence

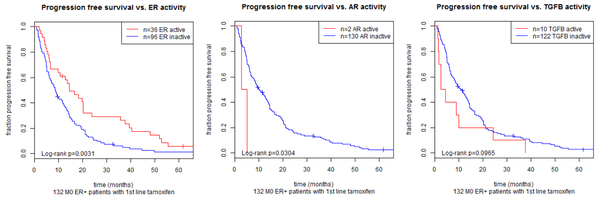
A multivariate Cox proportional hazards regression for progression free survival using transcriptional pathway activities, revealed that ER pathway activity is significantly associated with favorable PFS, while AR and TGFβ activity are marginally associated with a shorter PFS.



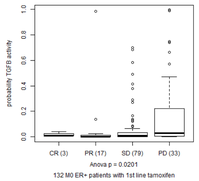
When combining ER pathway activity with traditional risk prediction factors, it remains statistically significant, next to disease free interval and ESR1 microarray expression (although the latter has little variation).



A Kaplan-Meier analysis confirms the association of ER, AR and TGFβ pathway activity to PFS (logrank p = 0.003, 0.03 and 0.097, respectively).

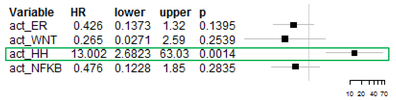


An Anova analysis of pathway activity across response groups (CR, PR, SD, PD) again revealed that the probability of TGFβ pathway activity as calculated by our models is higher in patients with SD and PD.

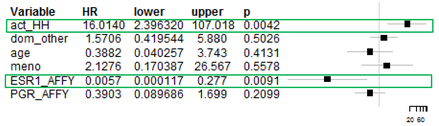


20 M1 patients

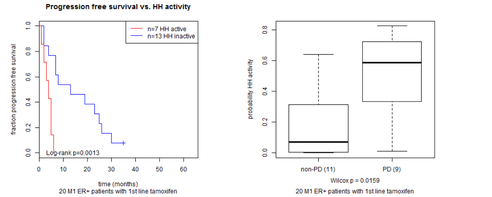
The 20 patients that already had metastases at presentation (M1), show a different picture than the M0 patients. None of these patients had an active AR, PI3K or TGFβ pathway. Of the other pathways, a multivariate Cox proportional hazards regression for PFS showed that HH activity was strongly associated with a shorter PFS.



When combining HH pathway activity with traditional risk prediction factors, it remains statistically significant, next to ESR1 microarray expression (although the latter has little variation).



A Kaplan-Meier analysis confirms the association of HH pathway activity to a shorter PFS (logrank p = 0.0013), while a Wilcox rank sum test of pathway activity on PD vs. non-PD patients again revealed that the probability of HH pathway activity as calculated by our model is higher in patients with PD.



References

[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;74(11):2936-45.

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