Assessing functional androgen receptor (AR) pathway activity using a computational model

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Introduction

Cellular signal transduction research identified 10-15 signaling pathways responsible for driving cancer growth. Assessing pathway activity in tumor tissue will help to optimize targeted therapy choice.

The androgen receptor (AR) pathway is responsible for driving growth of hormone sensitive prostate cancer (PCa). In presence of androgens, AR forms a homodimer and translocates to the nucleus, initiating the AR pathway's transcription program.

We developed a computational model to assess functional activity of the androgen receptor (AR) pathway in individual samples, based on mRNA expression levels of its target genes.

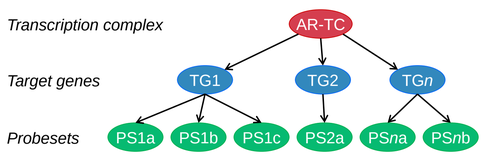
This model was successfully adapted for RT-qPCR input data, underscoring the portability of our approach to other gene expression measurement platforms.

Conclusions

- **The AR model was biologically validated in prostate cancer cell lines and cell line xenograft models. As expected, we found:**
  - **high AR pathway activity in presence of androgen, and**
  - **low AR pathway activity in absence of androgen or presence of anti-androgen.**
- **In prostate tissue samples, AR pathway activity**
  - **was highest in primary prostate cancer followed by hyperplasia and significantly lower in normal tissue, and**
  - **was lower in advanced (castrate-resistant prostate cancer and metastases samples) than in primary cancer, suggesting a role of other tumor-driving pathways in advanced prostate cancer.**
- **In other cancers, AR pathway activity was mostly low, except for a subset of HER2 and Luminal A subtype breast cancer.**

AR Pathway model (Affymetrix)

We built a Bayesian network model of the AR transcriptional program, which interprets the pathway target genes' mRNA levels (from Affymetrix HG-U133 arrays) and infers a probability that the AR-pathway is active in a certain sample [1].



- The model describes (i) how the expression of the AR target genes depends on the activation of the AR transcription complex, and (ii) how probeset intensities depend in turn on the expression of the respective target genes.
- The Affymetrix AR pathway network is comprised of 28 AR target genes and was calibrated on data from PCa LNCaP and DUCaP cell line experiments with known activity status, by either depriving them from dihydrotestosterone (DHT), or stimulating them with DHT.

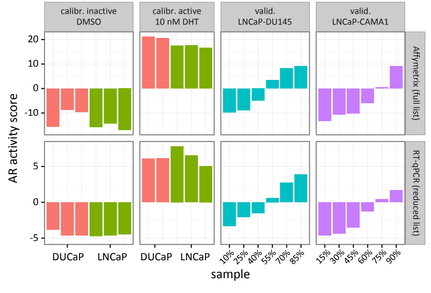
[1] W. Verhaegh et al. Selection of personalized patient therapy through the use of knowledge-based computational models that identify tumor-driving signal transduction pathways. Cancer Res 2014;74(11):2936-45.

[2] E. Lim, et al. Importance of breast cancer subtype in the development of androgen receptor directed therapy. Curr Breast Cancer Rep 2014;6(2):71-8.

Adaptation to RT-qPCR

We adapted the AR pathway model to RT-qPCR expression data using a subset of most informative AR target genes. This model was calibrated on RT-qPCR data from the same samples used for calibration of the Affymetrix model. Initial validation was done by comparing the inferred activities obtained by the Affymetrix model and the RT-qPCR model for:

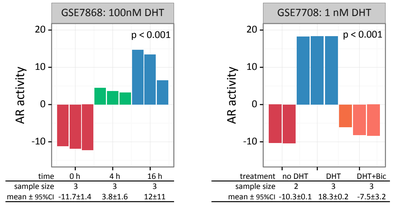
- the cell line samples used for model calibration, and
- a series of mixtures containing an increasing proportion of RNA from the AR active LNCaP cell line and RNA of an AR inactive cell line (either DU145 or CAMA1).



The good correlation obtained between the two models underscores the portability of our approach to other gene expression measurement platforms.

Pearson correlation	
All samples	97.7%
Validation samples only	96.7%

In-vitro LNCaP model



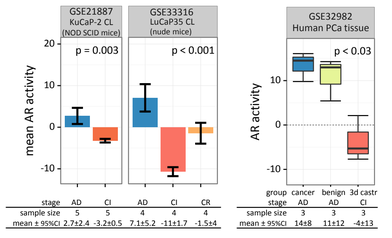
Inferred AR activity

- increased with exposure time, and
- decreased with anti-androgen bicalutamide treatment.

(p values from one-way ANOVA)

In-vivo models

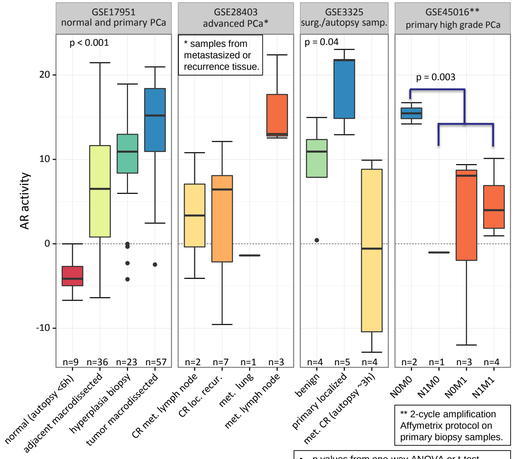
Cell line (CL) xenografts | Human PCa tissue



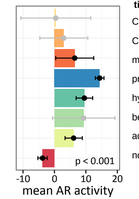
In each dataset, the AR model clearly separated the 3 PCa stages: androgen dependent (AD) growth, castration induced (CI) regression, and castration resistant (CR) regrowth. The difference between AD and CR stages remained significant when combining the 3 datasets, ANOVA $p < 0.001$.

(p values from one-way ANOVA, error bars indicate standard deviation)

AR activity in prostate tissue



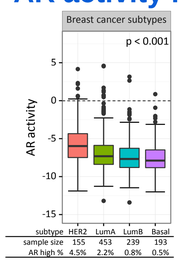
Combined PCa datasets:



tissue	n	AR high %
CR met.	6	50%
CR loc. recur.	7	71%
metastasis	11	82%
primary	65	97%
hyperplasia	23	83%
benign	4	100%
adjacent	36	78%
normal	9	0%

- Activity was significantly higher in
  - primary PCa than in normal and adjacent normal tissue,
  - hyperplasia than normal tissue,
  - adjacent normal tissue than in normal tissue.
- In cancer tissue, mean activity in advanced PCa (castrate-resistant and metastasized tissue) was significantly lower than in primary tissue, and had a larger spread.

AR activity in other cancer types



In other cancers, AR pathway activity was mostly low, except for a subset of HER2 subtype breast cancer (BCa) and Luminal A BCa (EM-TAB-365, GSE12276, GSE17907, GSE21653). In the combined dataset, AR activity in HER2 samples was significantly higher than in the other 3 subtypes, and activity in Luminal A samples was significantly higher than in the Basal subtype. This is in accordance to the current assumption that AR plays a role in HER2 BCa (as promoting role) and in Luminal A BCa (as tumor suppressing) and that AR activation is rare in basal like cancers [2].

In other cancer types, we found occasional high AR activity, e.g., 6 of 97 (6.2%) in meningioma (GSE16581, GSE9438), 14 of 313 (4.5%) in lung (GSE28571, GSE19804, GSE14017, GSE2109), and lower numbers in the lymph system, blood and colon.

(p value from one-way ANOVA, 22 of 1062 BCa samples (2%) removed after QC)

Materials and methods

- Calibration data of Affymetrix models and cell line mixtures used in PCR vs Affymetrix comparison was obtained using Affymetrix HG-U133+PM array plates.
- Affymetrix validation data was obtained from Affymetrix HG-U133Plus2.0 datasets deposited in the GEO and Ensembl databases.
- Affymetrix data was normalized using adapted fRMA normalization that is suitable for both HG-U133+PM and U133Plus2.0 arrays.
- All datasets used in this analysis (including public) passed though a QC procedure, based on Affymetrix recommendations. Samples that did not pass QC standard were removed.
- A default threshold of 0 was chosen for high/low dichotomization of AR activity.
- Statistical analysis was carried out in R and Minitab.