Molecular tests to determine the functional activity of tumor-driving signal transduction pathways

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Introduction

Signal transduction pathways regulate normal cell functions like cell division, migration and differentiation. However, aberrant activity of these pathways may cause cancer. Approx. twelve major signaling pathways play a role in tumor growth and metastasis among which: estrogen and androgen receptor (ER, AR) pathways, phosphoinositide 3-kinase (PI3K), activated by multiple growth factor receptors like HER2, EGFR, canonical Wnt, Notch, Hedgehog and TGFß pathways. Measurement of the mutations or changes in protein levels do not necessarily indicate whether the pathway is active. We developed a new approach to assess functional pathway activity by measuring the expression of the target genes of the pathways transcription factor complexes. This is used as input for a computational model that assesses the pathway activity.

Conclusion

Here we demonstrated a new tool for the application of therapy response prediction and monitoring in cell lines, PDX models and cancer patients**.**

- Both Affymetrix Array expression data as well as RT-qPCR expression data could be used as input, enabling measurement on FF as well as FFPE.
- The results show that our RT-qPCR test and our Affy based test were able to measure the activity of signal transduction pathways in various tumor types.

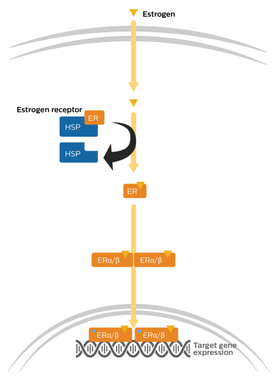
With more and more drugs in the pipeline that target signal transduction pathways in cancer this could be an important aid in the choice of personalized treatment.

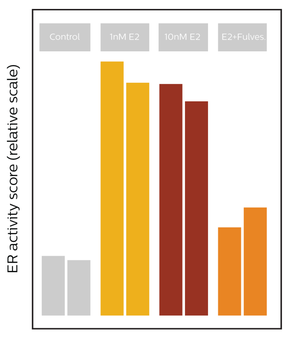
Results

MCF7 breast cancer cell line

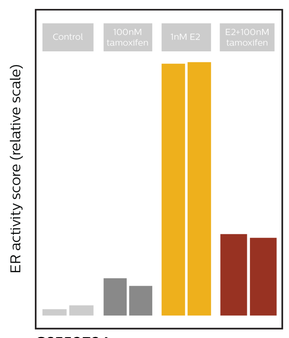
ER Pathway

ER positive cells with and without the addition of estrogen and subsequent addition of the selective estrogen receptor degrader (SERD) Fulvestrant or the selective estrogen receptor modulator (SERM) Tamoxifen.





A high ER pathway activity is measured after addition of Estradiol (E2) and a low pathway activity after subsequent inhibition by Fulvestrant (left) and Tamoxifen (right).



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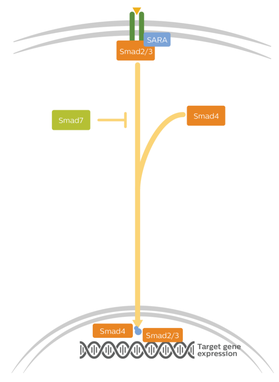
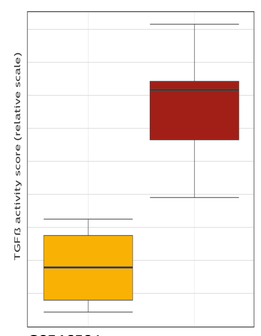
Glioblastoma stem cells

TGFß Pathway

Glioblastoma radiation-treated stem cells with or without temozolomide (cytostaticum).





A higher TGFß pathway activity is measured in treatment resistant cells.

GSE46531

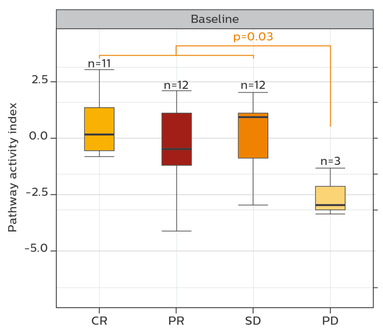
FFPE and FF clinical samples

ER Pathway — Neo-adjuvant hormonal breast cancer TEAM IIa study

Leids Universitair Medisch Centrum

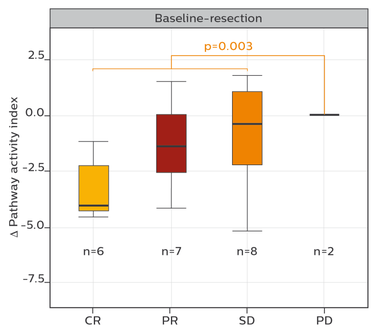
Baseline ER pathway activity measured in the diagnostic biopsy sample of ER positive post-menopausal breast cancer patients prior to treatment [1].

ER pathway activity measured in the diagnostic biopsy sample prior to treatment and on the corresponding surgical specimen after 3-6 months of treatment with Exemestane (Aromatase inhibitor) [1].



Progressive disease (PD) assessed by palpation is associated with low baseline ER pathway activity.

CR complete response
PR partial response
SD stable disease
PD progressive disease



Response to hormonal therapy is correlated to decrease in pathway activity.

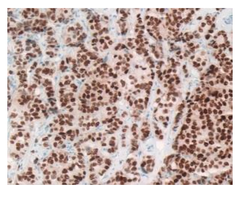
CR complete response
PR partial response
SD stable disease
PD progressive disease

ER stain positive breast cancer patients

ER activity measurement on the initial tumor of ER positive breast cancer patients prior to treatment to predict response to Tamoxifen [2].

Current ER subtyping

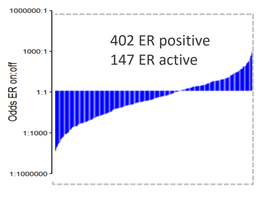
ER receptor stain positive (ER+)



>1% ER positive

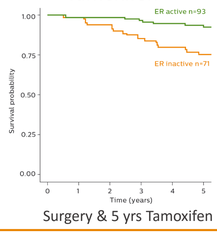
OncoSignal Pathway test

In ~40% of patients ER pathway is active



Response data

ER pathway active patients respond much better to Tamoxifen



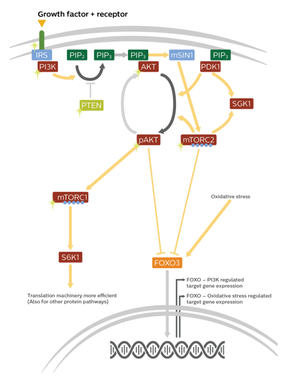
Surgery & 5 yrs Tamoxifen

ER active patients respond much better to Tamoxifen, an ER pathway inhibitor.

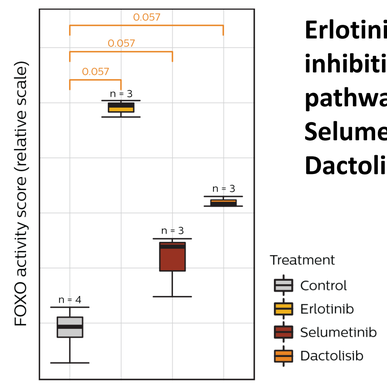
HCC877 lung cancer cell line

PI3K Pathway

FOXO activity score is inversely related to PI3K pathway activity!



Lung cancer cell line HCC877 with EGFR mutation treated with Erlotinib (EGFR inhibitor), AZD6244 (Selumetinib, inhibitor of MEK1/MEK2) or BEZ235 (Dactolisib, dual inhibitor of PI3K/mTOR).



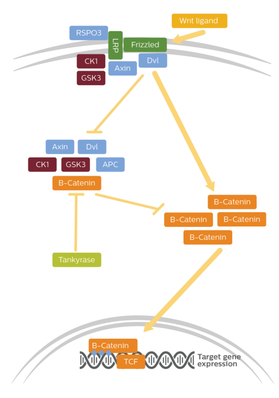
Erlotinib shows more inhibition of PI3K pathway activity than Selumetinib or Dactolisib.

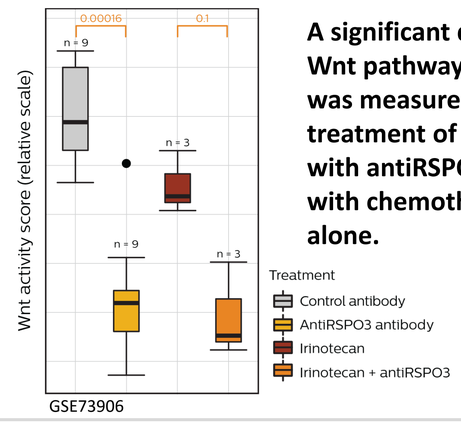
GSE51212

Colon cancer PDX mice

Wnt Pathway

Colon cancer PDX mice treated with antiRSPO3 (Wnt inhibitor) and/or chemotherapy.





A significant decrease in Wnt pathway activity was measured after treatment of PDX mice with antiRSPO3 and not with chemotherapy alone.

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Materials and methods

Known target genes of the transcription factors of AR, ER, FOXO, TGFß, HH, Notch and Wnt pathways were identified and computational models were developed for data interpretation linking gene expression to the presence of the related transcription factor complex and related pathway activity. After measuring the gene expression in a single model system with known pathway activity, the computational model was calibrated with these data and frozen. Publicly available data sets (Affymetrix HG-U133Plus2.0 or PM arrays) on cell line experiments and fresh frozen (FF) clinical samples were analyzed to initially validate our approach.

To enable pathway activity measurement in both FF as well as FFPE tissue from various cancer types and cell lines and to enable standardization of the measurement, 96 RT-qPCR assays for the most relevant pathways were developed. This RT-qPCR plate can be used on standard laboratory equipment and was used a.o. for the TEAM IIa study and several cell line experiments showing that the computational models can be ported to several measurement methods.

RNA was isolated using standard methods for FF or FFPE RNA isolation.

References:

[1] ER pathway activity as a predictive biomarker for neo-adjuvant endocrine therapy: Results of the TEAM IIA trial; EJ Blok et al, SABCS 2015

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

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