Assessment of functional signal transduction pathway activity in patient-derived tumor xenografts to predict and evaluate therapy response

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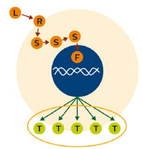
Summary

- **We developed and biologically validated a novel approach to assess functional activity of signal transduction pathways in individual tumor samples from target gene mRNA levels.**
- **Our pathway activity assessment is validated on samples from cell lines, tumor xenograft models and patients with known pathway activity status judged by their driver mutations and responses to pathway inhibitors.**
- **Pathway analysis adds functional information to mutation analysis in terms of altered signaling pathway activity, and hence can be used to assess functional consequences of mutations.**
- **Changes in pathway activity can be used to assess response to a targeted treatment.**
- **Conclusion: Functional signaling pathway activity measurement can be used to select the most appropriate PDX models in drug testing, and to predict and assess therapy response on a biological level.**

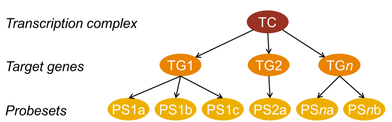
Knowledge-based models to measure signaling pathway activity

We developed knowledge-based computational models to quantitatively assess AR, ER, PI3K-FOXO, Hedgehog (HH), NFκB, TGFβ and WNT signal transduction pathway activity from mRNA expression levels of their respective direct target genes in cancer tissue [Verhaegh et al., Cancer Res 2014; 74(11): 2936-45]. We modeled the pathways in a probabilistic manner, using a Bayesian network, with three types of nodes: a transcription complex, target genes and probesets.



Testing 'down-stream' for mRNA transcription of a pathway's target genes

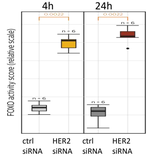
Each model describes (i) how the expression of the target genes depends on the activation of the respective transcription complex, and (ii) how probeset intensities depend in turn on the expression of the respective target genes. The models can be used to assess transcriptional pathway activity in a given test sample by entering its Affymetrix probeset measurements, and inferring backwards the odds for the presence of the transcription complex, i.e. that the pathway is active. These odds are represented on a logarithmic scale (log2odds), and/or translated to a score on a scale from 0 to 100.



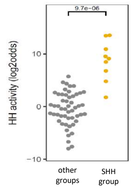
The network structure of the used Bayesian models

Biological validation

Models have been biologically validated first on data sets with ground truth information about pathway activity status.



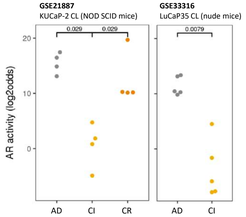
PI3K-FOXO activity following HER2-siRNA knockdown in breast cancer cell line BT-474 from GSE71347.
Note: FOXO inactive means PI3K active



HH activity in medulloblastoma from GSE37418.
The SHH group was identified by others as being marked by SHH signaling.

Biological validation on xenograft models

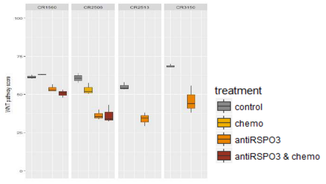
Subsequently, we fine-tuned the pathway models for tumor xenograft data and tested them on data sets from xenografts with known pathway activity status.



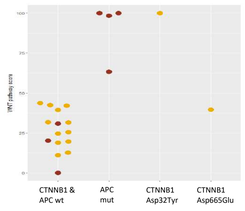
AR pathway activity in prostate cancer PDX models KUCaP-2 and LuCaP35 grafted in immunodeficient mice:

- high in androgen dependent PDX (AD)
- reduced in PDX undergoing castration-induced tumor regression (CI)
- high during regrowth of castration resistant tumors (CR)

GSE73906 colon cancer PDX



Charles River PDX collection (breast & ovary)



WNT pathway activity in colon cancer PDXs:

- higher in control and chemo-treated mice
- reduced by treatment directed against the WNT pathway component RSPO3.

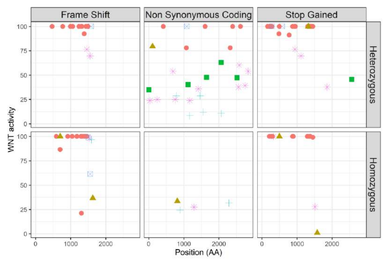
WNT pathway activity in ovarian (brown) and breast cancer (yellow) PDXs:

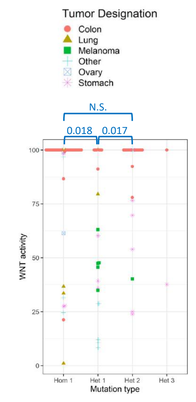
- low in wild type APC & CTNNB1 PDXs
- high in loss-of-function APC mutated (ovarian)
- high in known pathogenic CTNNB1 Asp32Tyr mutated (breast)
- low in non-annotated CTNNB1 Asp665Glu mutated (breast); gain-of-function unlikely

Wnt pathway activation by different APC mutants

The APC protein is a component of the WNT signaling pathway and negatively regulates β-catenin, which acts as part of the WNT transcription factor complex.

- APC frameshifts and stop mutations generally activate WNT signaling
- Homozygous, non-synonymous mutations are often found in non-colon tumors not displaying elevated WNT signaling





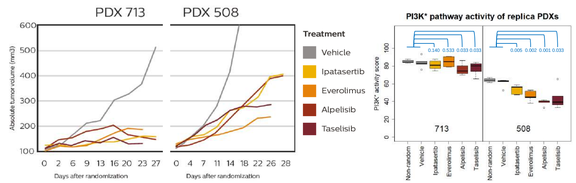
- Homozygous mutations result on average in higher WNT activity than heterozygous mutations
- Two heterozygous mutations in combination are more often associated with elevated WNT activity than one alone

Signaling pathway activity analysis provides insight into the functional consequences of a mutation

Charles River breast cancer PDX models with high PI3K* activity respond to PI3K-targeted therapy

PDX model	group	PI3K* activity	ER activity	observation	EGFR	ERBB2	PIK3CA	PIK3CB	PIK3R1	PTEN	ESR1
713	Breast	67.3	55.3	PI3K & ER high	wt	wt	wt	wt	wt	gene loss	wt
508	Breast	59.4	16.4	PI3K high	wt	wt	wt	wt	gene loss	gene loss	wt
2499	Breast	55.9	22.9		wt	amplified	wt	wt	wt	wt	wt
574	Breast	50.2	5.7		wt	Asp1058Ala	wt	wt	wt	gene loss	wt
2500	Breast	48.0	15.6		wt	amplified	wt	wt	wt	wt	wt
583	Breast	45.5	20.6		wt	wt	wt	wt	wt	wt	wt
857	Breast	41.8	12.1		wt	wt	wt	wt	wt	wt	wt
449	Breast	40.9	12.1		wt	wt	wt	wt	wt	wt	Glu542Val
MX1	Breast	38.6	9.8		wt	wt	wt	wt	wt	deletion	wt
1162	Breast	36.4	11.7		wt	amplified	wt	wt	gene loss	wt	wt
401	Breast	34.8	9.7		wt	gene loss	wt	wt	gene loss	gene loss	wt
1398	Breast	34.5	50.2		wt	wt	wt	wt	wt	Arg130Gly	Tyr537Asn
1384	Breast	31.5	26.7		wt	wt	wt	wt	wt	wt	wt
1322	Breast	28.3	15.9		wt	wt	wt	wt	wt	deletion	wt

Two breast cancer PDX models with highest PI3K* activity were selected for testing PI3K inhibition, and showed reduced growth upon treatment, even though they are wild type *PIK3CA*. Furthermore, PI3K* activity was lower in PI3K-inhibited PDX tumors from alpelisib- and taselisib-treated mice as compared to tumors from untreated and vehicle treated mice.

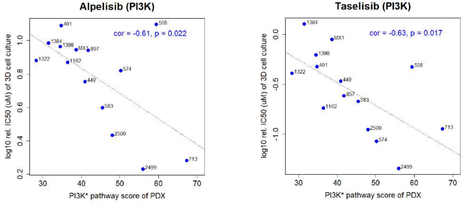


p-values from one-sided Wilcox rank-sum test

PI3K* activity is related to PI3K inhibition response in 3D ex-vivo PDX cell cultures

PDX models with a higher PI3K* activity (based on microarrays) show increased sensitivity (lower IC_{50} values) to PI3K inhibitors alpelisib and taselisib in 3D ex-vivo cultures.

This is not the case for mTOR and AKT inhibitors (data not shown).



*** The PI3K reading is derived from the inverse activity reading of the FOXO transcription factor.**
Please be aware that oxidative stress can induce FOXO activity, which may inadvertently lead to a low PI3K activity reading.

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