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Determination of signal transduction pathway activity in patient-derived xenograft models in comparison with clinical patient tumor samples for a variety of human cancer types

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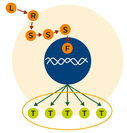
Summary

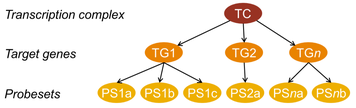
- **We developed and biologically validated a novel approach to assess functional activity of signal transduction pathways in individual tumor samples from target gene mRNA levels.**
- **Our pathway activity assessment is also validated on samples from xenograft models with known pathway activity status judged by their driver mutations and responses to pathway inhibitors.**
- **Pathway activity is quite stable across successive PDX passages.**
- **Pathway activity distributions and profiles are generally similar in patient tumors and PDX models.**
- **In individual cases, pathway scores may vary between patient tumors and respective PDX models, due to differences in the tumor microenvironment.**
- **Conclusion: With PDX models exhibiting specific pathway activity profiles, measuring pathway activities may be highly valuable for the selection of the most appropriate PDX models in drug testing, and as a predictor of therapy response.**

Knowledge-based models to measure signaling pathway activity



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

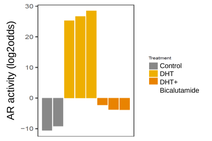
We developed knowledge-based computational models to quantitatively assess AR, ER, PI3K-FOXO, Hedgehog (HH), NFκB, TGFβ and WNT signal transduction pathway activity from cancer tissue mRNA expression levels of their respective direct target genes, measured on Affymetrix HG-U133Plus2.0 microarrays (fRMA preprocessed) [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. 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 an individual test sample by entering its Affymetrix probeset measurements, and inferring backwards what the odds are that the transcription complex must have been present, i.e. that the pathway is active. These odds are represented on a logarithmic scale (log2odds), which subsequently may be translated to a score on a scale from 0 to 100.



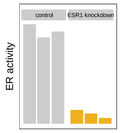
The network structure of the used Bayesian models

Biological validation

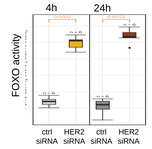
Models have been biologically validated first on data sets with ground truth information about pathway activity status.



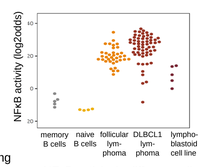
AR (androgen receptor) activity in LNCaP cell lines stimulated with DHT and blocked with bicalutamide from GSE7708.



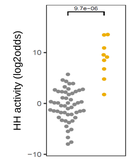
ER (Estrogen receptor) activity on ER positive MCF7 cell lines with and without ESR1 knockdown by siRNA from GSE37820



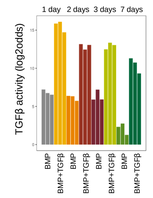
PI3K-FOXO activity following HER2-siRNA knockdown in breast cancer cell line BT-474 from GSE71347.
Note: FOXO inactive means PI3K active



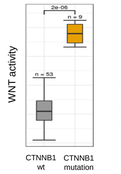
NFκB activity in different cell types from GSE12195.



HH activity in medulloblastoma from GSE37418.



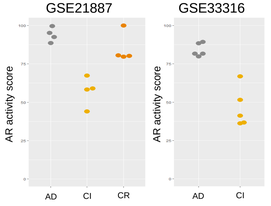
TGFβ activity in mesenchymal stem cells treated with BMP or BMP+TGFβ (1,2,3,7 days) from GSE84500.

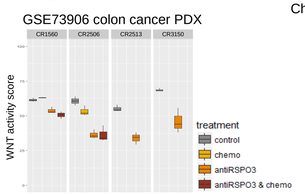


WNT activity in medulloblastoma without and with driving CTNNB1 mutation from GSE10327.

Biological validation on xenograft models

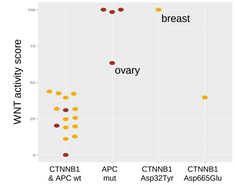
Subsequently, we fine-tuned the pathway models for xenograft data and tested them on data sets from xenografts with known pathway activity status.





WNT pathway activity in colon cancer PDX is
- higher in control and chemo-treated mice
- reduced by treatment directed against the WNT pathway component RSPO3.

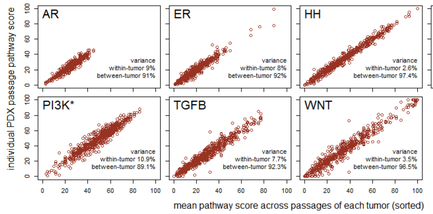
AR pathway activity in prostate cancer PDX models KUCaP-2 and LuCaP35 grafted in nude/immunodeficient mice:
- high in AD: androgen dependent
- reduced in CI: castration-induced tumor regression
- high again in CR: castration resistant tumor regrowth.



WNT pathway activity in ovarian (brown) and breast cancer (yellow) PDXs from CRL is
- low in APC & CTNNB1 wild type
- high in loss-of-function APC mutated ovary
- high in WNT activating CTNNB1 Asp32Tyr mutated breast
- low in non-functional CTNNB1 Asp665Glu mutated breast

Consistency across PDX passages

89 – 97% of the variation in pathway scores is due to differences between PDX models, and only 3 – 11% results from differences between passages of the same PDX model (GSE78806 with n = 661 PDX samples).

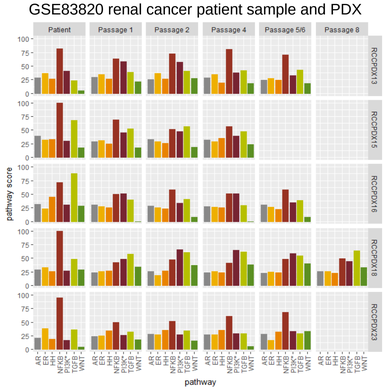


n = 127 one passage
n = 205 two passages
n = 32 three passages
n = 7 four passages

Patient tumors vs. PDX models

Comparing PDXs of different tumor types in the Charles River PDX collection to patient tumors in the expO compendium GSE2109, we generally see similar distributions of pathway activity.
Please note:
- There are only very few prostate cancer PDXs.
- Breast cancers include hormone-dependent and independent tumors. The proportion of hormone-dependent PDXs is low.
- The stroma of PDXs is of mouse origin. Pathways active in tumor infiltrates (e.g. often NFκB) cannot be assessed with human-specific methods.

Functional pathway activity can be used to select the most appropriate PDX models for drug testing and to interpret results of efficacy tests.

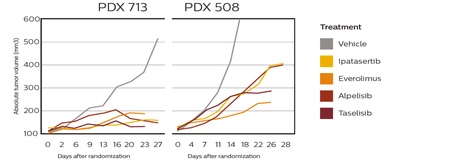


Patient renal cell cancers and matched PDX models in different passages from GSE83820 reveal generally comparable pathway activity patterns, with some exceptions:
- NFκB activity is well preserved in PDX passages of tumor 13, but not in tumor 18.
- TGFβ activity seems lost in PDXs of tumor 16.

Charles River PDX collection compared to GSE2109

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	45.3	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	wt	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



*** 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.

PHILIPS

charles river