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Measuring functional signal transduction pathway activity on breast cancer tissue samples to determine intra-tumor heterogeneity and heterogeneity between primary and metastatic tumors

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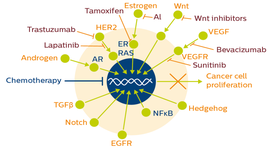
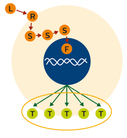
Summary

A novel *biologically validated* method to quantitatively measure activity of oncogenic signal transduction pathways was used to measure in breast cancer intra-tumor signaling pathway heterogeneity and heterogeneity between primary and metastatic tumors, revealing major heterogeneity between primary and metastatic tumors.

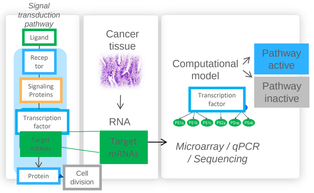
Knowledge-based models for quantitative measurement of signal transduction pathway activity



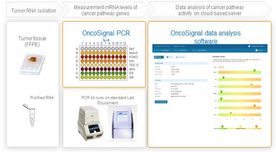
Testing "down-stream" for mRNA transcription of the target genes of pathways



Bayesian computational Models infer signal transduction pathway activity from mRNA expression levels of direct target genes of the pathway-associated transcription factor. Input for the measurement is Affymetrix HG-U133Plus2.0 microarray data or qPCR [Verhaegh et al., Cancer Res 2014;74(11):2936-45]. The network model has three types of nodes: a transcription complex, target genes and probesets. The 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.

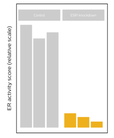


Translation from Affymetrix to qPCR plate

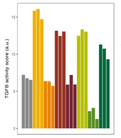


Models can be used to quantitatively measure pathway activity in an individual test sample by entering mRNA measurements, and inferring backwards in the model the probability (or log2 odds) that the active transcription complex must have been present.

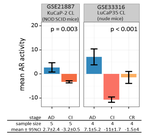
Pathway model calibration on one cell type; biological validation on different cell types (examples)



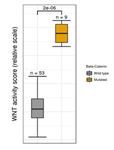
ER (Estrogen receptor). Calibration: Breast cancer cell line. GSE37820, ER knock down by siRNA in ER positive MCF7



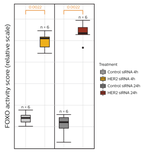
TGFbeta. Calibration: lung cancer cell line. GSE84500, Mesenchymal stem cells treated with TGFbeta



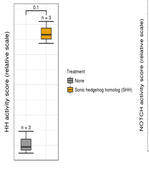
AR. Calibration: prostate cancer cell line. GSE21887, KUCaP-2 cell grafted in NOD SCID mice, androgen dependent tumor growth (AD) and castration-induced tumor regression (CI); GSE33316, LuCaP35 cells grafted in nude mice, AD, CI and castration resistant (CR) regrowth



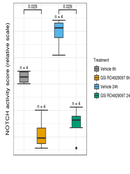
Wnt, Calibration: colon cancer cell line. GSE10327, medulloblastoma with beta-catenin activating mutation.



FOXO-PI3K. Calibration: HUVEC. GSE71347, HER2-siRNA knock down in breast cancer cell line (BT-474)



HH. GSE29316, Hedgehog (SHH) treatment of fibroblasts



Notch. Calibration: ovarian cancer. GSE6495, ALL MOLT4 cell line treated with γ-secretase inhibitor

Methods

Sample set 1: RNA from spatially distributed cancer tissue samples: (a) multiple biopsies per resected primary breast cancer; (b) multiple samples per biopsy block.

Sample set 2: RNA from matched primary and metastatic samples from different organ locations.

Pathology classification:

Class	ER	PR	HER2
Luminal A	+	+ or -	-
Luminal B	+	+ or -	overexpressed
HER2	-	-	overexpressed
Triple Neg	-	-	-

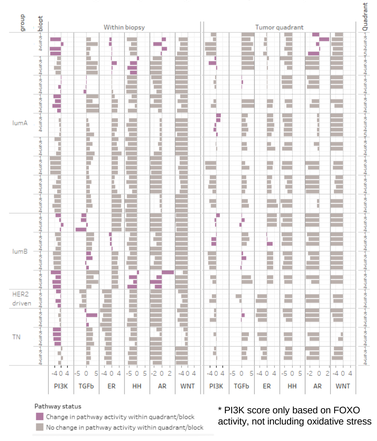
AR, ER, PI3K, HH, TGFbeta and Wnt pathway activity was measured using qPCR-based computational pathway models

- Pathway activity is indicated as log2 odds of the calculated probability.
- PI3K pathway activity was derived from FOXO transcription factor activity in combination with SOD2 gene expression to separate *growth control*- from *oxidative stress*-induced FOXO activity [poster SABCS 2017, publication under review].

Intra-tumor pathway heterogeneity

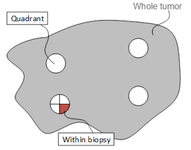
Heterogeneity across pathway activity threshold

Heterogeneity in log2 odds pathway activity



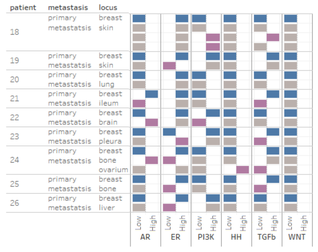
* PI3K score only based on FOXO activity, not including oxidative stress

	Luminal A (N=9)		Luminal B (N=4)		Triple Neg (N=3)		HER2 (N=1)	
	block	quadrant	block	quadrant	block	quadrant	block	quadrant
Estrogen receptor (ER)	2	-	1	1	-	-	-	-
Androgen receptor (AR)	1	1	1	-	-	-	-	-
PI3K	3	2	2	2	1	-	1	-
Hedgehog (HH)	1	-	1	-	-	-	-	-
TGFbeta	1	1	2	2	1	1	-	-
Wnt	-	-	-	-	-	-	-	-
1 or more pathway switches per block/quadrant	4 (44%)	4 (44%)	4 (100%)	3 (75%)	2 (67%)	1 (33%)	1 (100%)	0 (0%)

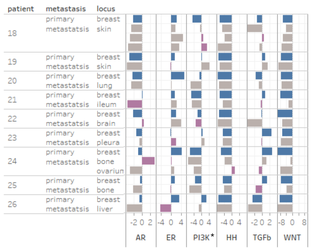


Pathway heterogeneity between primary and metastases; luminal breast cancer

Heterogeneity across pathway activity threshold



Heterogeneity in log2 odds pathway activity



* PI3K score only based on FOXO activity, not including oxidative stress

N=9 patients luminal breast cancer	Active in primary	Loss of activity in at least 1 meta	Inactive in primary	Gain of activity in at least 1 meta
Estrogen receptor (ER)	8	5 (62%)	1	1 (100%)
Androgen receptor (AR)	1	1 (100%)	8	1 (12%)
PI3K	2	1 (50%)	7	2 (28%)
Hedgehog (HH)	0		9	1 (11%)
TGFbeta	3	3 (100%)	6	2 (33%)
Wnt	0		9	0 (0%)
1 or more pathway switches between primary and metastases	8 (89%)			

Conclusion

Limited heterogeneity in signal transduction pathway activity was found within a biopsy block, more heterogeneity between quadrants of the whole tumor, and between primary tumor and metastases, as well as between metastases from the same patient.

Heterogeneity was lowest in the ER pathway and in Luminal A tumors; most heterogeneity was found in the PI3K pathway (if oxidative stress was also considered) and in more agressive breast cancer subtypes between primary and metastasis.

Results suggest that homo-/heterogeneity within a single biopsy is often representative for the whole tumor. Targeted drug treatment of metastatic breast cancer may require analysis of multiple biopsies to choose the most effective drug or drug combination.

PHILIPS

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