Heterogeneity in Signaling Pathway Activity within Primary Breast Cancer and between Primary and Metastases

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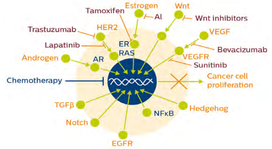
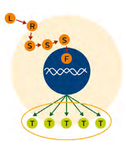
Summary A method to measure quantitative activity of oncogenic signal transduction pathways was used to assess molecular phenotype heterogeneity within primary tumors on a micro- and macro-scale, and between primary tumors and lymph node or distant metastases.
Pathway activity variances were analyzed using linear mixed models with subgroup-dependent standard deviations.

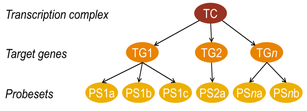
Quantitative measurement of signal transduction pathway activity





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

Knowledge-based computational models were developed to measure signal transduction pathway activity based on observed mRNA expression levels of direct target genes of the pathway-associated transcription factor [1-5]. Input data is from Affymetrix HG-U133Plus2.0 microarrays or qPCR. These Bayesian models have three types of nodes: (a) transcription complex, (b) target genes and (c) probesets, and describe (i) how the expression of the target genes depends on the activation of the respective transcription complex, and (ii) how probeset intensities depend on the expression of the respective target genes.



The network structure of the used Bayesian models

qPCR based workflow

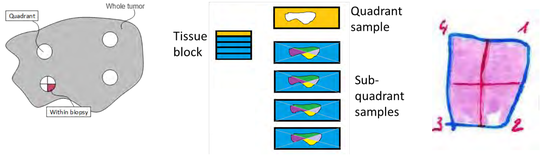
Models can be used to quantitatively measure pathway activity in an individual test sample by entering mRNA measurements, and inferring backwards the odds (on a log2 scale) that the pathway's transcription complex was activated.

* References pathway analysis: [1] Cancer Res. 2014;74(11):2936–45. [2] Oncotarget. 2014;5(14):5196–7. [3] Am. J. Pathol. 2018;188(9):1956–72. [4] Sci. Rep. 2019;9(1):1603. [5] Cancers. 2019;11(3):E293.

Breast cancer patients and sample sets

(I) Quadrant samples (macro-scale, Q): cancer tissue samples spatially distributed over the primary tumor: 15 luminal A, 9 luminal B, and 9 ER-negative.

(II) Subquadrant samples (micro-scale, SQ): 4 samples per quadrant tissue block: 9 luminal A, 4 luminal B, and 4 ER-negative.



(III) Matched primary-lymph node metastases from 7 patients: 7 primary and 24 lymph node samples.

(IV) Matched primary-distant metastases from 9 patients: 9 primary and 12 metastatic samples.

Breast cancer pathology classification

Breast cancers were classified based on immunohistochemistry

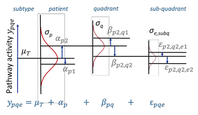
Class	ER	PR	HER2
Luminal A	+	+ or -	-
Luminal B	+	+ or -	overexpressed
HER2	-	-	overexpressed
Triple Neg	-	-	-

Measuring signaling pathway activity

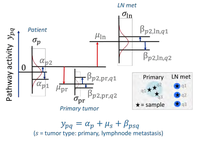
AR, ER, PI3K-FOXO, Hedgehog (HH), TGFβ and Wnt pathway activity scores were measured. PI3K pathway activity can be derived from FOXO transcription factor activity in combination with SOD2 target gene expression to separate *growth control*- from *oxidative stress*-induced FOXO activity [3].

Data analysis

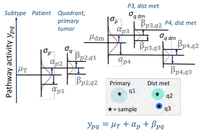
Quantification of heterogeneity in pathway activity was performed by analyzing variances in signaling pathway activity scores with linear mixed models with subgroup-dependent standard deviations.



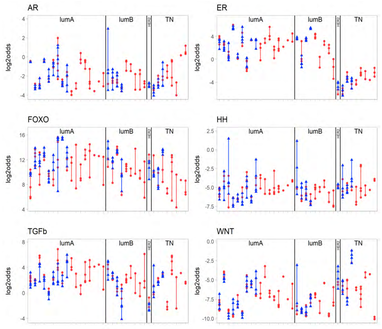
Calculation of variation (heterogeneity) in signaling pathway activity within the primary tumor to compare heterogeneity on a micro and macro scale within the primary tumor (sample set I and II)



Comparison of heterogeneity in pathway activity between primary tumor and multiple corresponding lymph node metastases (sample set Iii).



Comparison of heterogeneity in pathway activity between primary tumor and distant metastases (sample set IV)



● Quadrants ● Sub-Quadrants

Intra-tumor pathway activities

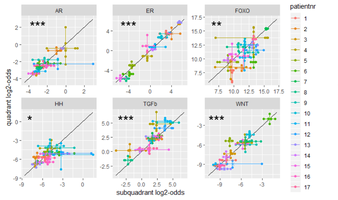
The left picture shows ranges of signaling pathway activity scores for quadrants and sub-quadrant samples; scales are relative for the patient population analyzed in this study.
For each individual patient, scores of corresponding (sub)quadrants are presented on vertical lines to illustrate their intra-patient ranges.

Pathway activity correlations

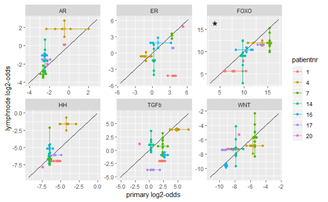
Pictures below and table right show correlations between pathway activity (averaged in case of multiple samples):

- strongest within primary tumors (quadrant vs. sub-quadrant samples),
- less between primary tumors and corresponding (spatio-temporally close) lymph node metastases,
- least between primary tumors and corresponding (spatio-temporally) distant metastases.

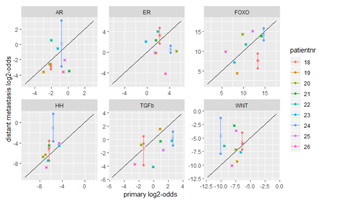
Pathway	Case	Correlation	pval (BFcorr)
AR	Q vs SQ	0.99	1.62E-12
ER		0.92	1.14E-06
FOXO		0.72	0.007
HH		0.68	0.015
TGFb		0.89	9.44E-06
WNT		0.92	7.98E-07
AR	Prim vs LN	0.88	0.056
ER		0.40	1.000
FOXO		0.92	0.023
HH		0.83	0.132
TGFb		0.31	1.000
WNT		0.65	0.704
AR	Prim vs DistMet	-0.01	1.000
ER		-0.36	1.000
FOXO		0.51	0.989
HH		0.60	0.522
TGFb		0.56	0.722
WNT		-0.16	1.000



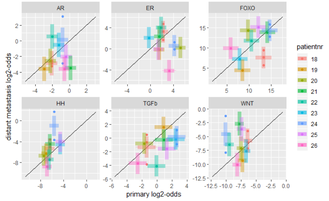
Quadrant vs. sub-quadrant activity



Primary vs. LN metastasis activity



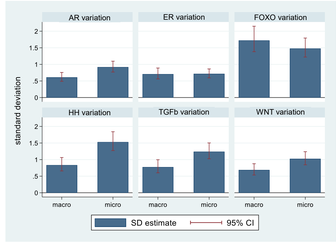
Primary vs. distant metastasis activity



Primary vs. distant metastasis activity (with model-predicted variances)

Intra-tumor heterogeneity

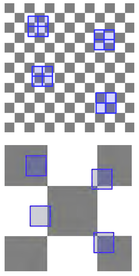
Within primary breast cancer, variation in activity of AR, ER, PI3K-FOXO, and Wnt pathways was not significantly different when measured on macro- (quadrant) or micro-scale (sub-quadrant). Variation in HH and TGFβ activity was significantly higher on a micro-scale (sub-quadrant) than macro-scale (quadrant; ratio = 1 rejected with $p < 0.001$). This may be explained by e.g. the presence of small sub-clones.



Intra-tumor pathway activity heterogeneity on macro-scale (Q) and microscale (SQ).

Primary-metastases heterogeneity

Variation in signaling pathway activity between distant metastasis (model predicted) is larger than between lymph node metastases ($p < 0.05$)



[top] Small sub-structures may give higher heterogeneity on a micro-scale (SQ) than macro-scale (Q)
[bottom] Large structures may give higher heterogeneity on a macro-scale.

Conclusion

Relative variation in signal transduction pathway activity within a primary tumor was generally modest and

- not significantly different on a macro- than microscale for ER, AR, PI3K-FOXO and Wnt pathways,
- higher on a microscale for the HH and TGFβ pathways,

suggesting that analysis of a single biopsy is generally sufficient for a phenotypic characterization of a primary tumor.

Micro-scale heterogeneity may be explained e.g. by the presence of small cancer stem cell clones, characterized by higher HH and TGFβ activity, which might confer therapy resistance, e.g. chemo-radiation.

PI3K-FOXO pathway activity in the primary tumor was indicative for activity in lymph nodes (spatially and temporally close), but not for activity in distant metastases (spatially and temporally distant). Hence it may be beneficial to take samples from metastases to decide on treatment strategy.