Determining tumor-driving cell signaling pathways in breast cancer: Support for targeted therapy selection

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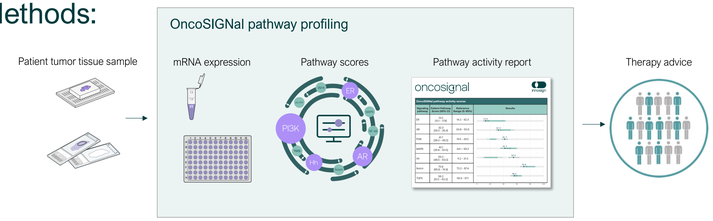
Introduction:

Success of therapeutic interventions largely rely on the pathological and biological characteristics of the tumor and varies due to the heterogeneous nature of breast cancers. Signal transduction pathways play an important role and are often used as target for specific therapies like hormone treatment, PI3K inhibitors etc. Immunohistochemistry (IHC) is currently the first assessment to divide tumors for subtyping and treatment and, especially in more advanced disease, mutation analyses are often used. Both IHC and mutation analyses only study a specific part of a signal transduction pathway and do not correlate well with outcome. Therefore, there is a need for new approaches to investigate signaling pathways in cancer. Here we show a new methodology that measures and quantifies signal transduction pathways (STPs) activities to reveal potential tumor-driving STPs to identify new options for targeted therapy in breast cancer. In previous studies we have shown that low ER pathway activity is strongly associated with poor response to endocrine therapy, despite of high ER-IHC percentages.

Methods:



Using the mRNA-based OncoSIGNal pathway activity profiling test (InnoSIGN), STP activities of 7 pathways (ER, AR, PI3K, MAPK, Hh, Notch, and TGFβ) were measured and quantified on a scale from 0-100.

Reference ranges for normal physiological pathway activity were defined based on epithelial breast tissue reference samples. A threshold for high activity was set on the 95th percentile of this range. If the complete confidence interval of a pathway activity score measurement of a patient tumor sample was above this threshold, the pathway activity was considered to be high (activated).

Using this methodology FFPE breast tumor samples obtained from different study sites and biobanks were analyzed, including samples from primary and metastatic lesions, to determine for each individual tumor its pathway activity profile and identify activated tumor-driving pathways. OncoSIGNal pathway profiling is available for clinical use in breast cancer through the InnoSIGN CLIA lab, Mason, OH.

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Results:

In 195 **primary ER-IHC positive tumors**, the ER pathway was found to be activated in only 77% of the samples, even though all samples were strongly (>50%) ER immunohistochemistry stain positive.

In all but one of the **primary ER-IHC negative tumors** (being HER2+ or TNBC tumors) the ER pathway was inactive as expected. An activated MAPK and/or PI3K pathway was found in the majority of the samples.

Occasionally other pathways were activated: e.g. in ER-IHC+ tumors, Hh in 27% and AR in 16% of the cases. In 8% of the primary tumors no activated pathway was detected.

PRIMARY	ER	AR	PI3K	MAPK	Hh	Notch	TGFβ	N
ER-IHC+ (10 HER+, 185 HER2-)	77%	16%	33%	15%	27%	1%	6%	195
ER-IHC- (12 HER+, 74 TNBC)	1%	10%	71%	44%	19%	3%	6%	86

Within the ER-IHC positive tumors, the ER pathway *inactive* tumors have more often an activated PI3K and/or MAPK pathway compared to ER pathway *active* tumors. This pathway profile resembles the profile of ER-IHC negative (TNBC) tumors.

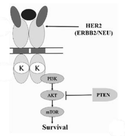
PRIMARY ER-IHC+	ER	AR	PI3K	MAPK	Hh	Notch	TGFβ	N
ER-IHC+	77%	16%	33%	15%	27%	1%	6%	195
ER-STP active	100%	19%	28%	14%	26%	1%	7%	151
ER-STP inactive	0%	8%	45%	23%	30%	0%	3%	44

Like for primary tumors, also for **metastatic tumors** the ER pathway is not always active despite of strong positive ER-IHC stain. Furthermore, metastatic ER-IHC+ tumors show more frequently high PI3K pathway activity (68% vs. 33%).

In ER-IHC negative cases, both metastatic and primary tumors have high PI3K pathway activity (71-75%). Metastatic ER-IHC- tumors show more frequently high Hh activity (38% vs. 19%); while MAPK is more frequently active in primary tumors (44% vs. 13%).

METASTASES	ER	AR	PI3K	MAPK	Hh	Notch	TGFβ	N
ER-IHC+	77%	5%	68%	5%	23%	0%	0%	22
ER-IHC-	0%	13%	75%	13%	38%	0%	0%	8

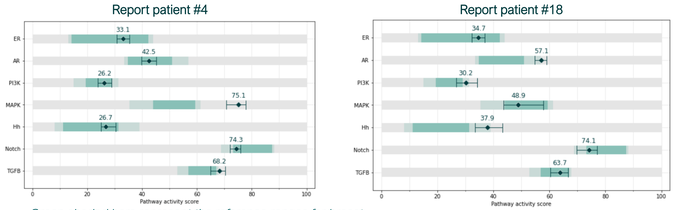
In **HER2 positive tumors** we found that the percentage of PI3K pathway active samples was higher as compared to ER+ HER2- tumors, as could be expected since HER2 is one of the receptors involved in the PI3K pathway. In **Triple negative** (TNBC) tumors PI3K and MAPK pathways also play a dominant role.



PRIMARY	ER	AR	PI3K	MAPK	Hh	Notch	TGFβ	N
HER2+ ER-IHC+	60%	10%	60%	0%	30%	0%	0%	10
HER2+ ER-IHC-	0%	8%	58%	42%	17%	0%	0%	12
HER2- ER-IHC- (TNBC)	1%	11%	73%	45%	19%	4%	7%	74
HER2- ER-IHC+	78%	17%	31%	16%	26%	1%	6%	185

OncoSIGNal patient pathway profiling

For each patient, a personalized pathway activity profile can be made to guide decision makers in choosing potential targeted therapy options. Example reports for two TNBC patients are shown here: Patient #4 has high MAPK activity, whereas patient #18 has high AR and Hh activity:



Green shaded bars represent the reference ranges for breast

Summary of actionable results for TNBC patients

Although many TNBC samples show high PI3K, MAPK, Hh and/or AR activity, the pathway profile is very different for each patient, providing a unique opportunity to select personalized targeted therapy based on the specific patient's pathway activity profile.

TNBC patient #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
ER																						
AR					■	■	■											■	■			
PI3K										■	■	■	■	■	■	■	■			■		
MAPK	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
Hh							■	■	■	■	■						■	■				
Notch											■											
TGFβ										■												

High pathway activity profiles per patient indicated in purple (>95th percentile of reference range for breast)

Conclusions:

- Not all ER-IHC positive patients have an activated ER pathway, explaining why not all patients respond to endocrine therapy.
- High PI3K pathway activity is frequently observed in the more aggressive breast cancers, including TNBC, HER2+ and metastatic tumors.
- High MAPK pathway activity is often observed in ER-IHC negative compared to ER-IHC positive tumors.
- Profiling of individual tumor samples reveals patient specific pathway activation patterns, demonstrating that OncoSIGNal can be used to determine the tumor-driving signaling pathways in breast cancer patients, guiding selection of personalized targeted therapies.

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