Development of quantitative multi-platform tests for easy readout of AR, ER, PI3K and MAPK pathway activity to unravel pathophysiology across cancer and tissue types

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Introduction:

Selection of targeted therapy is commonly based on genetic data, such as well-known gene mutations, but these mutations do not provide conclusive evidence for the functional activity of the affected signal transduction pathways. For this reason, we have developed tests to assess the molecular phenotype of individual tumors, determining the functional activity of oncogenic signaling pathways based on mRNA expression levels of the direct target genes of the respective pathway transcription factors. mRNA levels are translated via a computational model into quantitative pathway activity scores. (Verhaegh et al., Cancer Res 2014; Van Ooijen et al., Am J Pathol 2018; Van de Stolpe et al., Sci Rep 2019). Tests are developed to provide similar pathway activity scores when performed on RT-qPCR, RNA sequencing and microarray expression data. Presented results illustrate reproducibility of performance of the AR, ER, PI3K and MAPK pathway activity tests when performed simultaneously on a sample.

Material and Methods:

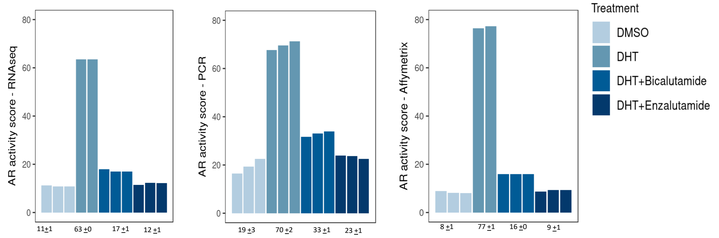
- PCR primers and probes were developed to assess mRNA expression levels of the pathways direct target genes for ER, AR, PI3K and MAPK.
- Per pathway, the same set of calibration samples and target genes were used to develop RT-qPCR, RNA sequencing and microarray tests, to maximize similarity.
- OncoSignal pathway activity is expressed using a scale from 0-100, where 0 being the lowest activity and 100 the highest activity the model can theoretically predict. Biological ranges may differ between pathways and tissue types.
- Verification performed on independent samples with known pathway status.
- Repeat experiments performed on cell-lines and human samples to assess the technical reproducibility within each platform.
- Pathway activity score concordance analyzed by Pearson correlation.

Results:

Measurement of pathway activity score using RNAseq, RT-qPCR or Affymetrix micoarray data, results in comparable outcomes.

For each of 4 pathways, samples were measured mostly in triplicates, under activated and non activated conditions.
As an example the AR pathway activity was measured on LNCaP cells after activation with DHT and inhibition of either Enzalutamide or Bicalutamide

- High concordance between different platforms for pathway activity scores
- High and low pathway activity clearly separated (19 vs 70 score) between control, activated and inhibited pathways
- Effect of different drugs on AR pathway activity inhibition can be measured

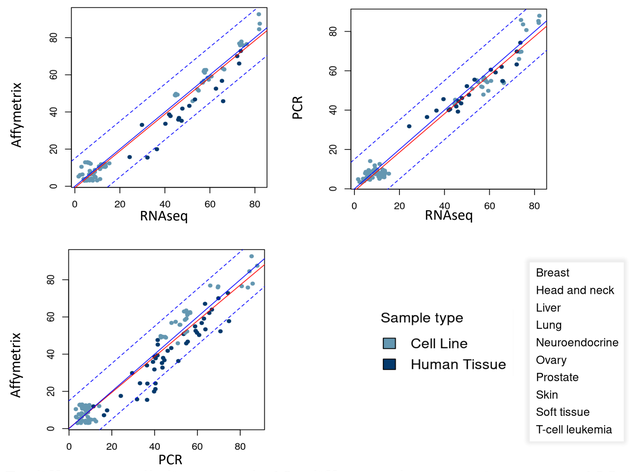


Similar experiments were performed for ER, PI3K and MAPK pathway tests. E.g. when performing PI3K pathway activity analysis on MCF-7 cells using Affymetrix microarray platform, a PI3K activity score of 88 ± 1 was found in samples before treatment and of 50 ± 4 after treatment with the PI3K inhibitor drug Alpelisib.

Total of 100+ data points (10 different tissue types and different cell lines) were measured, indicating that pathway activity outcome scores are highly comparable when tests are performed on different mRNA measurement platforms.

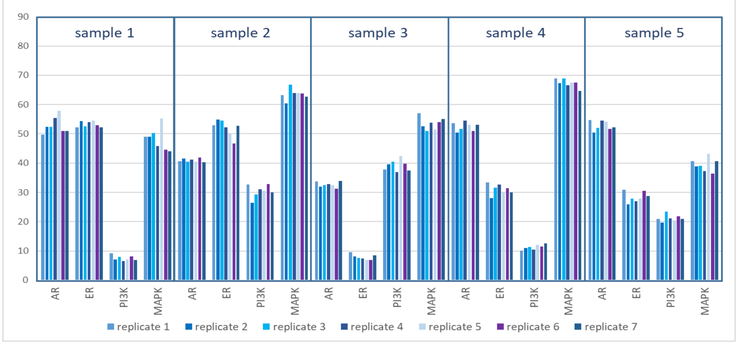
Pathway		RNA seq vs qPCR n=121	RNA seq vs Affy n=106	qPCR vs Affy n=132
ER	Pearson Correlation Samples within 10 points	0.98 97.5%	0.97 100%	0.93 100%
AR	Pearson Correlation samples within 10 points	0.95 91.7%	0.96 95.3%	0.94 88.6%
PI3K	Pearson Correlation Samples within 10 points	0.97 82.6%	0.98 95.3%	0.95 76.2%
MAPK	Pearson Correlation Samples within 10 points	0.99 95.9%	0.98 93.4%	0.96 90.2%

Pearson correlation (r) value for all 4 pathway activity test results performed on different mRNA measurement platforms. The percentage of samples that fall within an absolute pathway activity range of 10 pathway activity score points is also indicated.



7 different cell lines and 10 different human tissues (n=106-132) were measured for pathway activity scores on different mRNA measurement platforms. As an example the results of the MAPK pathway is shown. High concordance is observed between the platforms using cell lines and human samples of different origin.

Technical reproducibility of pathway activity measurements, resulting in a reliable test system to measure biological differences, even in tissue samples with highly degraded RNA (FFPE)



5 clinical FFPE tissues were scraped, RNA was extracted and measured 7 times using the qPCR OncoSignal test (suitable for FFPE).
Technical variation variables were: different analysis days, different operators, different PCR real-time machines.

Conclusion:

OncoSignal tests to simultaneously measure ER, AR, PI3K and MAPK activity in cell lines and human samples have been developed on three different mRNA measurement platforms; RNA seq, qPCR and Affymetrix. We demonstrate:

- High concordance between pathway tests performed using the different platforms and across tissue types
- Comparable quantitative functional signal transduction pathway activity scores on the same sample
- High reproducibility of the pathway activity scores in initial repeat experiments on qPCR plates using FFPE samples, showing that qPCR is a reliable method to quantify pathway activity scores in a single FFPE tumor tissue sample.

OncoSignal pathway activity tests can be used to unravel disease pathophysiology in (pre) clinical samples by evaluating simultaneously the signal transduction pathway activity profile of ER, AR, PI3K and MAPK pathways in one single tissue sample.

Multiple clinical studies are ongoing to demonstrate the value of OncoSignal tests for prediction of response to targeted therapies

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