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Summary

- The PI3K pathway is commonly hyper-activated in various types of cancer, but reliable predictive diagnostics for PI3K inhibitors is lacking.
- The PI3K pathway negatively regulates tumor suppressive FOXO transcription factors.
- We developed a computational model to assess *functional* activity of the PI3K-FOXO pathway in individual samples, using tissue mRNA expression data of FOXO target genes.
- Our model has been biologically validated on various cell lines with FOXO induction and PI3K inhibition, and tested on a large cohort of breast cancer patient samples.
- FOXO activity may be high while PI3K is active in case of oxidative stress, which we measure by SOD2 expression. This occurs most frequently in more aggressive breast cancer subtypes.
- Our functional PI3K pathway assessment may be highly relevant for PI3K therapy prediction.

Knowledge-based pathway model

We developed a knowledge-based computational model to infer transcriptional FOXO pathway activity from cancer tissue mRNA expression levels of its 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. 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.



The model can be used to estimate transcriptional FOXO activity in an individual test sample by entering its Affymetrix probeset measurements, and inferring backwards in the model what the probability is that the transcription complex must have been present.



Model calibration

Model parameters were calibrated using Human Umbilical Vein Endothelial Cells (HUVEC) with inducible constitutively active FOXO3.A3-ER from GSE16573. Samples before and after induction were used as FOXO inactive and active calibration samples, respectively.



HUVEC cell lines from GSE16573 with 40HT-inducible FOXO3.A3-ER expression construct were used for calibration (inside the blue box).

Biological validation

First biological validation was done on in-house experiments with doxycyclin inducible FOXO3.A3 constructs in MCF7 and MDA-MB-231 cell lines, showing correct assessment of FOXO activity.





cell line experiments.

Measuring functional PI3K pathway activity in cancer tissue using FOXO target gene expression in a diagnostic computational model

Inverse relation: FOXO inactive means

PI3K active

Western blot analysis of FOXO3 expression levels before induction and 16h after.

FOXO pathway activity in MCF7-FOXO3.A3



Immunofluorescent staining of FOXO3 (white in B/W images, yellow in overlay with blue DAPI).



MCF-FOXO3.A3 MCF-FOXO3.A3 + dox MDA-MB-231-FOXO.A3 MDA-MB-231-FOXO.A3 + dox

FOXO pathway activity in second induction experiment with MCF7 vs. MDA-MB-231

Cell line drug response

Applying our FOXO model on BT20, MDA-MB-453 and MCF7 experiments from GSE30516 showed a clear increase in FOXO activity upon EGFR inhibition with erlotinib.

In GSE16179, FOXO activity also increased after lapatinib (anti HER2) treatment of ER+/HER2+ cell line BT474. The increase is less on lapatinibresistant derived clone BT474-J4, but large again after restoring lapatinib sensitivity with foretinib.



Changes in FOXO activity upon erlotinib treatment.

FOXO activity in breast cancer Analyzing FOXO activity on a compiled set of public breast cancer data (n=1883), FOXO generally active in normal breast, normal-like breast cancer and luminal A breast cancer (89 - 96%) FOXO inactive in 34% of luminal B, Normal indicating PI3K activity NormL SOD2-high FOXO inactive in ~18% of HER2 and basal breast cancer FOXO activity can be restored by oxidative stress (see right text column): Normal Normal-like Lum A Lum B HER2 Oxidative stress (SOD2-high) increases • 5% of FOXO active luminal A

we observed:

with subtype aggressiveness:

- 71% of FOXO active basal



Changes in FOXO activity upon lapatinib treatment

■ FOXO inactive ■ SOD2-high FOXO active ■ SOD2-low FOXO active

Cellular oxidative stress

Literature suggests that FOXO can be activated by cellular oxidative stress, which is often associated with PI3K signaling. This may be assessed using expression levels of the FOXO target gene SOD2 (superoxide dismutase 2).



PI3K activity may lead to oxidative stress, thereby re-activating FOXO.

In breast, we assessed SOD2-overexpression by using expression in normal breast tissue as a reference, and taking the mean + 2 SD as a threshold. Affymetrix arrays have two probesets measuring SOD2; if both exceed their thresholds, we call a sample SOD2-high.



Thresholds for SOD2 probesets are based on normal breast tissue.

PI3K activity assessment tree

Using FOXO activity and SOD2 expression together gives the following scheme to assess PI3K pathway activity.



Decision tree for PI3K activity

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