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

PHILIPS innovation + you

[1] Philips Research, Eindhoven, The Netherlands
contact: anja.van.de.stolpe@philips.com

Henk van Ooijen[1] Marten Hornsveld[2] Christa Dam-de Veen[1] Rick Velter[1] Meng Dou[1] Wim Verhaegh[1] Boudewijn Burgering[2] Anja van de Stolpe[1]

UMC Utrecht

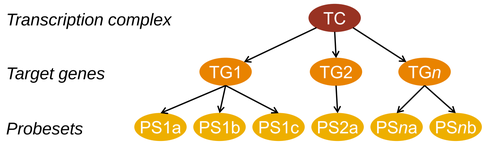
[2] UMC Utrecht, The Netherlands
contact: b.m.t.burgering@umcutrecht.nl

Summary

- **The PI3K pathway is commonly hyper-activated in various types of cancer, but tests to reliably predict response to PI3K inhibitors are lacking; mutation analysis has proven insufficient.**
- **An active PI3K pathway blocks FOXO transcriptional activity.**
- **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 can be restored in the presence of PI3K activity by oxidative stress, characterized by high expression of FOXO target gene SOD2. 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

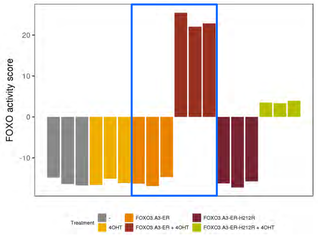
We developed a knowledge-based computational model to infer transcriptional FOXO pathway activity from cancer tissue mRNA expression levels of its direct target genes, measured on Affymetrix HG-U133Plus2.0 microarrays (fRMA preprocessed) [Verhaegh et al., Cancer Res 2014;74(11):2936-45]. We modeled the 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 P is that the transcription complex must have been present. This probability can be used directly, or first converted into a pathway activity score by calculating $\log_2(P / (1-P))$, which shows more detail if P is close to 0 or 1.

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.

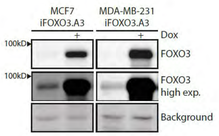


Inverse relation:
FOXO inactive
means
PI3K active

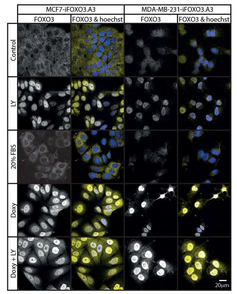
HUVEC cell lines from GSE16573 with 4OHT-inducible FOXO3.A3-ER expression construct were used for calibration (inside the blue box).

Biological validation

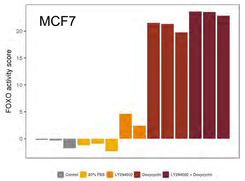
First biological validation was done on in-house experiments with doxycyclin inducible FOXO3.A3 constructs in MCF7 and MDA-MB-231 cell lines, showing correct assessment of FOXO activity.



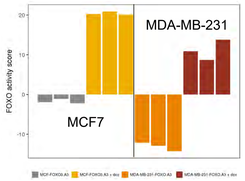
Western blot analysis of FOXO3 expression levels before induction and 16h after.



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



FOXO pathway activity in MCF7-FOXO3.A3 cell line experiments.



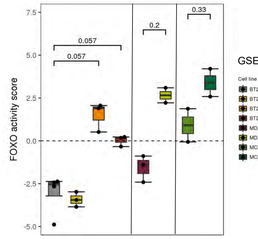
FOXO pathway activity in second induction experiment with MCF7 vs. MDA-MB-231.

Cell line drug response

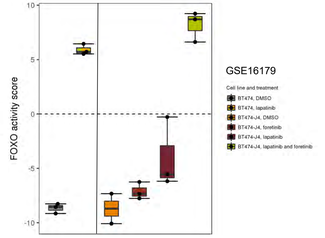
Also on public data sets, measured FOXO activity is as expected.

BT20, MDA-MB-453 and MCF7 experiments from GSE30516 showed a clear increase in FOXO activity upon EGFR inhibition with erlotinib (left picture).

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



Changes in FOXO activity upon erlotinib treatment.



Changes in FOXO activity upon lapatinib treatment.

FOXO activity in breast cancer

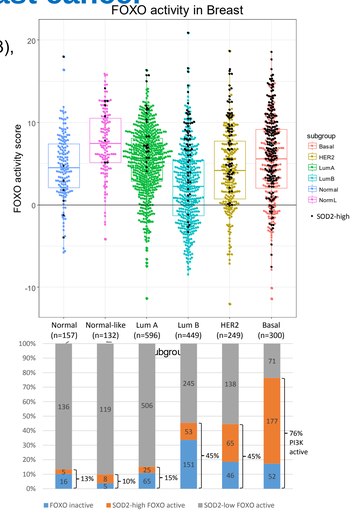
Analyzing FOXO activity on a compiled set of public breast cancer data (n=1883), we observed:

- FOXO generally active in normal breast, normal-like breast cancer and luminal A breast cancer (89 – 96%)
- FOXO inactive in 34% of luminal B, indicating PI3K activity
- FOXO inactive in ~18% of HER2 and basal breast cancer

FOXO activity can be restored in the presence of PI3K activity by oxidative stress (see right text column), characterized by high expression of FOXO target gene SOD2.

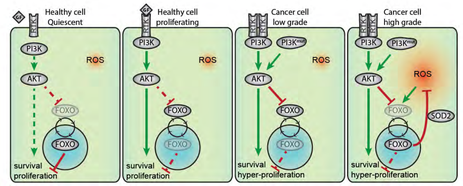
Oxidative stress (SOD2-high) increases with subtype aggressiveness:

- 5% of FOXO active luminal A
- 71% of FOXO active basal



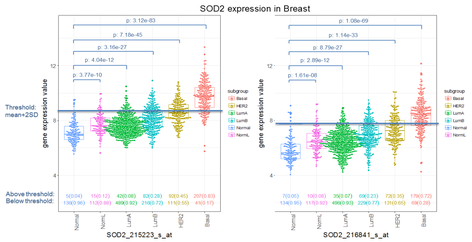
Cellular oxidative stress

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.

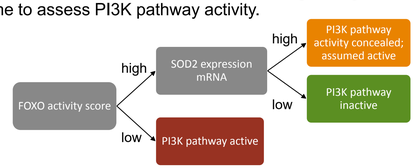
In breast, we assessed SOD2-overexpression by using expression in normal breast tissue as a reference, and taking the mean + 2 SD as a threshold. Affymetrix arrays have two probesets measuring SOD2; if both exceed their thresholds, we call a sample *SOD2-high*.



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 pathway activity

San Antonio Breast Cancer Symposium, December 5-9, 2017