

Assays for measurement of functional signal transduction pathway activity in any cell or tissue sample

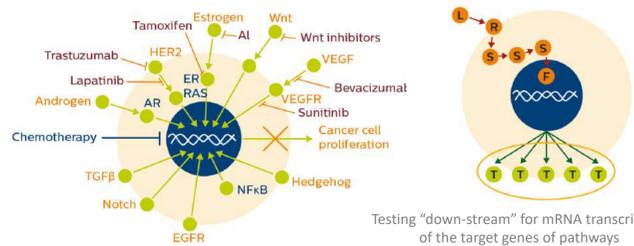


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Summary

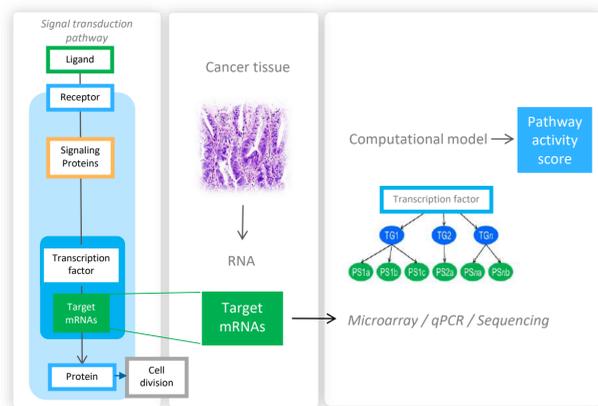
- A novel **biologically validated** method for quantitative measurement of activity of the ER, AR, FOXO-PI3K, Wnt, TGFbeta, Hedgehog, Notch, and NFkB signal transduction pathways in **any cell or tissue type**.
- Bayesian models interpret measurements of target mRNAs of pathway-associated transcription factors to provide activity scores.



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

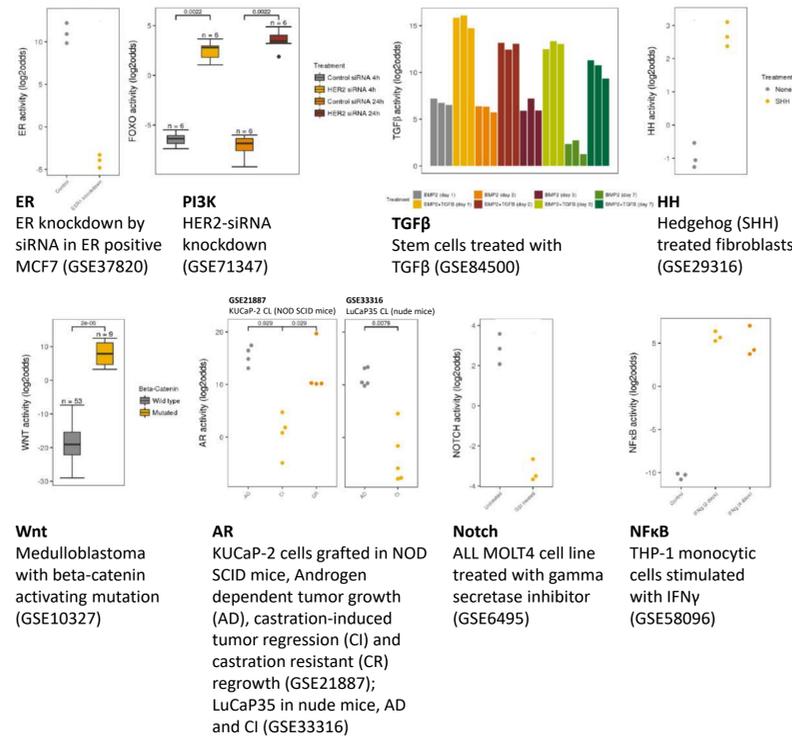
Assays for quantitative measurement of signal transduction pathway activity

Activity level of different signal transduction pathways is assessed by measuring mRNA expression of downstream target genes of pathway transcription factors using Affymetrix HG-U133Plus2.0 or RT-qPCR (for FFPE tissue). Knowledge-based Bayesian models translate mRNA expression levels into a quantitative pathway activity score as described in Cancer Res 2014;74(11):2936-45.



Biological validation of pathway models on different cell types

Pathway models are calibrated on one cell type. For each pathway one example on **another cell type** is shown below.



Pathway activity in human colon

Human colonic mucosa cells with different EPHB2 surface levels. Jung et al. found that low EPHB2 levels corresponded to differentiated, non-dividing cells (GSE31255, Nature Medicine 2011; 17 (10): 1225-7).

We find that Notch and WNT pathway activity are correlated to EPHB2 levels and can differentiate between stem cells and differentiated cells.

The proliferative EPHB2-high cells show increased PI3K-activity.

group	patient.id	AP1	ER	FOXO	HH	WNT	NOTCH
EPHB2 high	1	-5.6	-12.6	-4.9	-12.6	19.9	7.9
EPHB2 medium	1	-5.9	-12.4	-2.0	-13.1	-0.7	4.4
EPHB2 low	1	-3.8	-7.9	-0.6	-15.1	-13.0	3.1
EPHB2 neg	1	1.5	-5.0	5.0	-17.8	-22.8	1.9
EPHB2 high	2	-6.7	-12.1	-3.2	-11.4	21.7	8.7
EPHB2 medium	2	-5.5	-11.1	-3.4	-12.3	1.1	6.5
EPHB2 low	2	-5.9	-7.8	0.6	-14.0	-11.4	4.4
EPHB2 neg	2	2.0	0.6	3.9	-17.4	-23.6	5.4
EPHB2 high	3	-3.5	-11.0	-2.7	-10.8	27.4	8.8
EPHB2 medium	3	-3.2	-12.3	-2.2	-11.4	10.6	7.3
EPHB2 low	3	1.2	-9.7	-0.7	-14.9	-4.7	4.7

Pathway activity in human stem cells

Human embryonic stem cells (HES) cultured under different conditions (GSE19902). Active HH pathway.

Activity of TGFbeta, Notch and PI3K pathway activity depend on culture conditions.

Original data: BMC Cell Biol 2010 Oct 12;11:76

group	FOXO	HH	TGFbeta	Wnt	NOTCH	STAT3
hES-T3 cells grown on feeder-free Matrigel in T3HDF-conditioned medium	-0.6	13.8	0.4	-15.2	5.9	3.1
hES-T3 cells grown on feeder-free Matrigel in T3HDF-conditioned medium	0.8	13.9	1.6	-14.9	6.8	3.3
hES-T3 cells grown on feeder-free Matrigel in MEF-conditioned medium	-11.8	14.1	-13.8	-19.1	-1.3	2.2
hES-T3 cells grown on feeder-free Matrigel in MEF-conditioned medium	-11.9	14.6	-13.5	-18.8	-1.7	1.9
hES-T3 cells grown on T3HDF feeder	-9.6	16.6	-3.2	-18.2	9.3	6.6
hES-T3 cells grown on T3HDF feeder	-9.9	15.5	-0.3	-17.7	8.2	6.5
hES-T3 cells grown on MEF feeder	-11.4	14.7	-15.8	-14.5	-3.8	2.3
hES-T3 cells grown on MEF feeder	-11.4	14.8	-16.6	-14.8	-3.7	2.6

Differentiation of human stem cells to endoderm (GSE52658)

Human embryonic stem cells (hESC) can be differentiated into various cell-types under the control of specific signals. Signaling pathway activity is a readout of such signals and measuring pathway activity can be a useful tool to assess the differentiation/maturation status of cultured cells.

Loh et al. differentiated hESCs into various cell types (GSE52658) and reported on the required signaling molecules (in green). We measured signaling pathway activity for each cell type, resulting in specific pathway signatures (interesting pathways in orange).

Human embryonic stem cells

group	FOXO	HH	TGFbeta	Wnt	NOTCH	STAT3
Human embryonic stem cells, undifferentiated	-15.9	11.7	-1.6	-11.5	4.7	2.6
Human embryonic stem cells, undifferentiated	-16.1	12.0	-1.0	-12.1	6.4	3.5
Human embryonic stem cells, undifferentiated	-15.8	11.4	-3.6	-11.9	5.5	3.8

Anterior primitive streak

group	FOXO	HH	TGFbeta	Wnt	NOTCH	STAT3
Anterior primitive streak (SR1-induced), day 1	-12.7	9.3	-5.7	-6.1	3.8	2.3
Anterior primitive streak (SR1-induced), day 1	-12.7	9.5	-7.2	-4.9	3.1	2.7
Anterior primitive streak (SR1-induced), day 1	-13.3	9.7	-6.2	-6.4	3.2	2.1

Definitive endoderm

group	FOXO	HH	TGFbeta	Wnt	NOTCH	STAT3
Definitive endoderm (SR1-induced), day 3	-6.5	5.6	-5.1	-12.0	3.4	-1.9
Definitive endoderm (SR1-induced), day 3	-7.3	5.7	-9.1	-11.7	2.2	-1.6
Definitive endoderm (SR1-induced), day 3	-6.9	6.4	-7.1	-12.7	2.7	-1.2

Mesoderm

group	FOXO	HH	TGFbeta	Wnt	NOTCH	STAT3
AFBly-differentiated hESCs, day 3	-5.9	7.0	-2.1	2.3	3.6	-2.1
AFBly-differentiated hESCs, day 3	-6.3	7.5	-4.8	3.0	3.1	-2.2
AFBly-differentiated hESCs, day 3	-6.2	6.4	-3.9	1.7	3.2	-1.6

Original data: Cell Stem Cell. 2014 Feb 6;14(2):237-52

Anterior foregut (lungs, thyroid)

group	FOXO	HH	TGFbeta	Wnt	NOTCH	STAT3
Anterior foregut (SR1-induced), day 7	-7.1	15.9	-9.0	-14.7	1.0	3.3
Anterior foregut (SR1-induced), day 7	-9.1	14.3	-8.9	-16.2	2.8	0.9
Anterior foregut (SR1-induced), day 7	-7.0	15.5	-9.7	-14.8	1.7	1.3

HH

BMP↓, TGFbeta↓

Posterior foregut (pancreas, liver)

group	FOXO	HH	TGFbeta	Wnt	NOTCH	STAT3
Posterior foregut (SR1-induced), day 7	-5.0	16.1	-5.4	-13.2	3.6	1.4
Posterior foregut (SR1-induced), day 7	-3.9	15.0	-6.5	-13.5	3.3	1.9
Posterior foregut (SR1-induced), day 7	-5.8	17.3	-6.2	-13.2	2.7	1.7

HH, (NOTCH), (FOXO)

BMP↓, RA↓

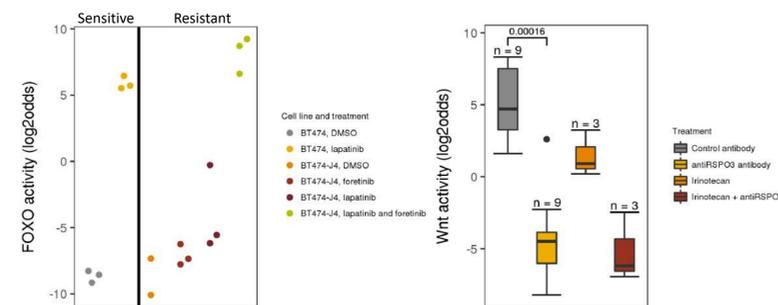
Midgut/hindgut (intestines)

group	FOXO	HH	TGFbeta	Wnt	NOTCH	STAT3
Midgut/hindgut (SR1-induced), day 7	-13.6	17.3	-12.8	2.0	7.3	1.6
Midgut/hindgut (SR1-induced), day 7	-13.8	16.8	-12.3	2.9	6.1	1.0
Midgut/hindgut (SR1-induced), day 7	-12.7	17.2	-12.2	2.4	7.2	1.8

HH, NOTCH

BMP↑, FGF↑, Wnt↑

Quantifying drug response in cell culture and PDX mice



Effect of PI3K inhibitors on FOXO-PI3K pathway activity. BT474-J4 cell line is lapatinib-resistant (GSE16179)

Colon cancer PDX mice treated with antiRSP03 (Wnt inhibitor) and/or chemotherapy (Irinotecan) (GSE73906)