Analysis of active oncogenic signal transduction pathways in HGS ovarian cancer

HH pathway activity associated with platin resistance

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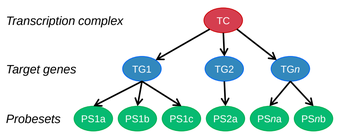
Introduction

Patients with ovarian cancer in general have a bad prognosis due to frequently advanced disease at diagnosis associated with limited therapeutic options, and recurring disease. Use of targeted drugs in ovarian cancer is not very advanced, one major reason being insufficient knowledge on tumor driving signaling pathways. Hence there is a strong need for a better diagnostic approach.

Signal transduction pathway analysis approach

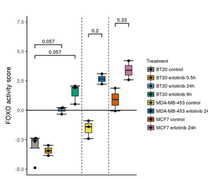
A new diagnostic approach is being developed [Verhaegh et al. Cancer Res 2017;74(11):2936-45] that enables quantitative measurements of the functional activity status of (oncogenic) signal transduction pathways in cell and tissue samples, based on computational inference of pathway activity from measurements of *mRNA levels of well-validated direct target genes of the transcription factor* associated with the respective signalling pathway. mRNA levels can be measured using Affymetrix HG-U133Plus2.0 or +PM microarrays or RT-qPCR.



Validation examples of pathway models

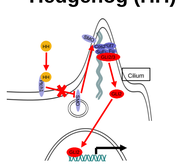
PI3K-FOXO

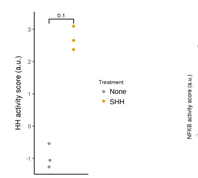
Note: FOXO activity is the inverse of PI3K activity



GSE30516: Eroltinib (EGFR inhibitor) inhibits PI3K pathway activity

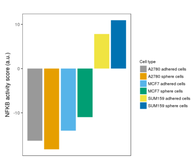
Hedgehog (HH)





GSE29316: Sonic Hedgehog induces HH pathway activity in fibroblasts

NFkB



GSE43657: NFkB activity in inflammatory breast cancer cell line SUM159

Materials and methods

Affymetrix HG-U133+PM microarrays were run on fresh frozen samples from 37 primary high grade serous (HGS) ovarian cancer patients (90 samples in total, from multiple metastatic locations). These data, and data from a number of publicly available Affymetrix datasets were analysed for signal transduction pathway activity, including Hedgehog (HH), PI3K and NFkB pathway activity.

Results

HGS ovarian cancer patient samples, Erasmus MC

Two common pathway patterns were found:

- NFkB+FOXO active, i.e. PI3K inactive
- PI3K+Hedgehog (HH) active

Patient 17, primary carboplatin/paclitaxel resistant HGS ovarian cancer, with rapid disease progression and death: HH active and PI3K active (FOXO inactive)

Pt nr	grade	FIGO	POS (prim. debulk. surg.)	Chemotherapy	NDS (interval debulking surgery after	PD event	Time to PD (months)	Death event	Time to death (months)	loc	%T	FOXO	HH	NFkB	PI3K inact NFkB act	PI3K act HH act
1	3	IC	Yes, incomplete	3x CtxPi/T + 2x C/T	no	1	36.6	1	53.9	Adnex left	80	12.0	-3.9	2.5	X	
										Ovarium	90	3.2	-5.1	-10.4		
2	3	IIIC	yes, incomplete	C/T	no					Ovarium	70	5.6	-6.3	-7.2		
										Omentum	30	9.4	1.6	3.7	X	
3	3	IIIC	yes, incomplete	CisPt/T 5x +2x C/T	yes, rest < 1 cm	1	16.3	1	45.6	Omentum	35	11.5	-0.4	10.0	X	
										Ovarium right	70	12.6	1.7	8.9	X	
4	3	IIIC	yes, complete	4x CtxPi/T	no	0	102.0	1	102.0	Adnex	40	1.3	6.3	10.0	X	
5	3	IIIC	yes, incomplete	C/T 6x 3 w	no	1	54.0	1	81.7	Ovarium	45	13.3	-3.2	12.3	X	
										Ovarium	90	15.1	-4.6	10.7	X	
6	3	IV	yes, incomplete	C/T 9x 3 w	yes, complete	1	18.0	1	40.7	Omentum	35	17.6	-0.9	8.9	X	
										Ovarium	100	14.4	-4.4	14.6	X	
										Ovarium	50	15.8	-4.9	12.6	X	
										uterus	85	9.5	-4.0	12.1	X	
7	2	III	yes, suboptimal	C/T x9	no	1	10.6	1	20.8	Omentum	60	7.3	5.8	11.1	X	
8	3	IIIB	yes, optimal	6x C/T	no	1	31.3	1	120.1	Ovarium	75	10.9	2.5	-7.7		
9	3	IIIC	yes, incomplete	3x C/T+ postop elsewhere	yes, complete				21.7	Ovarium	80	4.2	4.5	-11.2		
										Ovarium	90	1.9	3.8	-8.3		
10	3	IIIC	yes, optimal	C/T	no	1	11.5	1	13.5	Omentum	75	4.9	-6.0	-0.4		
										Omentum	70	7.1	-6.5	0.5	X	
										Omentum	80	6.8	-6.3	1.0	X	
										Ovarium	75	6.0	-6.7	4.2	X	
11	nk	IIIC	yes, optimal	6 C/T	no	0	133.8	0	133.8	Omentum	85	12.9	-0.3	9.8	X	
										Omentum	85	6.6	-2.5	-10.5		
										Ovarium	90	3.1	-2.3	1.9	X	
										Ovarium	90	1.2	-1.8	-3.4		
12	3	IV	yes, optimal	6x C/T	no	1	17.8	1	41.2	Ovarium left	70	18.0	-6.4	-1.0		
										Ovarium right	55	19.7	-4.1	10.2	X	
13	1-2	IIIC	yes, incomplete	6x C/T + 3x C/T	yes, optimal	1	21.0	1	84.7	Ovarium	80	1.6	5.3	-14.7		
										Ovarium	80	3.5	3.7	-9.7		
										Ovarium	85	6.2	1.9	-12.4		
										Ovarium	80	4.9	1.8	5.8	X	
										Ovarium	85	1.5	1.5	4.6	X	
										Ovarium	70	5.0	-1.2	7.0	X	
14	3	IIIC	yes, optimal	9x C/T 3wkl	no	1	27.4	1	61.8	Adnex left	70	8.3	-4.2	-6.0		
										Adnex right	80	10.1	1.5	-9.4		
										Omentum	50	7.3	-4.8	-0.8		
										Rectum	55	8.6	4.1	6.8	X	
										Uterus	40	5.0	7.6	9.8	X	
15	2	IIIC	yes, optimal	6x C/T	no	1	77.5	0	103.9	Ovarium	70	2.7	3.8	2.3	X	
16	2	IIIB	yes, optimal	no	no			1	1.2	Ovarium	70	2.1	-7.0	2.3		
17	3	IIIC	yes, complete	6 x C/T	no	1	6.5	1	11.1	Omentum	95	6.4	4.3	-9.7		X
										Omentum	90	4.0	3.5	-11.8		X
										Omentum	95	9.2	3.3	-11.1		X
										omentum	95	4.4	2.9	-11.3		X
										Omentum	90	6.3	2.5	-12.7		X
										Omentum	95	7.6	1.9	-12.0		X
										Omentum	95	4.1	1.0	-12.7		X
										Omentum	95	5.3	0.4	-12.0		
										ovarium	95	8.3	6.1	-13.0		X
										Ovarium	95	8.8	5.6	-13.3		X
										Ovarium	90	9.3	3.6	-14.4		X
18	2	IIIC	yes, suboptimal	9 x C/T	yes, complete	1	14.4	1	69.9	Ovarium	90	0.2	8.9	-1.7		
19	nk	IV	yes, incomplete	3+3 x C/T	yes, complete	1	49.2	1	75.1	Omentum	50	7.9	12.6	16.8	X	
20	2	IV	yes, incomplete	6x w C/T + 4x C/T then IOS	yes, complete	1	7.5	1	8.1	Ovarium	70	7.9	6.5	-5.7		
										Ovarium	65	1.5	6.4	-9.8		
										Ovarium	85	8.1	0.4	-6.1		
										Ovarium	75	8.8	2.0	-4.0		
22	3	IIIB	yes, complete	C/T	no			1	20.2	Ovarium	75	23.1	1.3	9.2	X	
										Ovarium	70	20.1	3.9	5.6	X	
23	3	IIIC	yes, complete	C/T	no	1	25.6	1	41.4	Ovarium	60	7.4	8.6	10.6	X	
										Ovarium	90	13.4	4.0	4.1	X	
24	3	IIIB	yes, inoperable	3 x C/T + 6x C/T	yes, complete	1	20.3	1	78.7	Omentum	80	4.9	1.7	1.9		
										Omentum	80	2.9	4.4	7.5		
										Omentum	80	5.8	6.5	0.5		
25	2	IV	yes, incomplete	6x w C/T	no			1	2.0	Ovarium left	80	1.0	1.3	9.4		
26	3	IIIC	yes, optimal	9 x C/T	no	1	37.7	1	50.3	Ovarium	50	20.1	1.9	15.8	X	
										Ovarium	50	10.5	1.1	6.9	X	
27	3	IIIC	yes, complete	6 x w C/T	no	1	83.5	0	76.1	Adnex left	40	14.5	0.8	9.1	X	
										Adnex right	45	3.1	6.2	1.4	X	
28	3	IV	yes, incomplete	6x w C/T + 6 x C/T + Tarceva (erlotinib)	no	1	16.0	1	52.2	Ovarium	45	16.2	0.1	10.6	X	
										Ovarium	60	11.9	3.1	12.7	X	
										Ovarium	65	16.4	1.7	15.0	X	
										Ovarium	70	16.2	4.3	12.9	X	
29	2	IIIC	yes, incomplete	6x w C/T + 6x C/T	no	1	19.0	1	65.6	Omentum	70	7.6	11.0	3.9		
30	2	IV	yes, optimal	9x C/T	no	1	29.0	1	52.5	Omentum	65	10.1	5.1	9.2	X	
										Omentum	70	7.2	5.6	8.1	X	
31	2	IC	yes, complete	no refusal pat	no	1	111.6	0	212.2	Adnex	70	16.4	6.2	5.5	X	
										Adnex	50	16.2	7.2	4.0	X	
32	3	IIIC	yes, incomplete	3x C/T + 6x C/T after IOS	yes	0	73.2	0	73.2	Ovarium right	95	12.4	0.4	9.8	X	
										Ovarium right	80	14.0	2.1	11.7	X	
33	2	IIIC	yes, optimal	C/T	no	1	11.2	1	37.4	Omentum	50	11.5	0.7	8.5	X	
										Ovarium	80	11.8	4.7	16.2	X	
34	nk	IIIC	yes, complete	6x C/T	no			0	76.8	Ovarium	70	11.5	3.5	-6.5		
35	2	IIIC	yes, suboptimal	7x w C/T + 3x C/T	yes, complete	1	28.5	1	28.8	Peritoneum	50	3.7	-1.6	13.9	X	
36	3	IIIC	yes, suboptimal	6 x C/T	no	1	54.2	0	71.8	Omentum	45	10.3	4.1	10.8	X	
39	3	IIIC	yes, incomplete	3x C/T +2x C/T after IOS	yes, complete	1	20.7	1	61.3	Ovarium	85	16.6	1.7	8.7	X	
										Ovarium	65	17.2	2.2	12.6	X	

Histology	1	Serous/HGS
FIGO		FIGO stage
PDS		primary debulking surgery
IDS		interval debulking surgery
P		primary-untreated disease (biopsy)
Rec		recurrence
Chemotherapy	C/T	Carboplatin/Paclitaxel
	CisPt/T	Cisplatin/Paclitaxel
	PD	progressive disease
	nk	not known

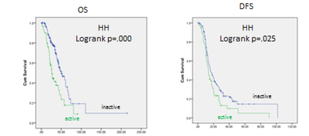
Public (GEO) datasets HGS

array	sample	FOXO	HH	NFkB	PI3K inact NFkB act	PI3K act HH act
GSM797484	516	7.7	-4.0	18.6	X	
GSM797485	518	6.9	-9.5	8.7	X	
GSM797486	519	-6.6	-4.2	2.7		
GSM797487	520	8.7	-1.9	15.9	X	
GSM797488	521	9.0	-7.3	11.0	X	
GSM797489	522	-0.2	-1.6	10.8		
GSM797490	523	-4.2	8.9	-3.6		X
GSM797491	525	-1.9	0.2	12.9		X
GSM797492	526	17.4	-5.4	25.4	X	
GSM797493	527	20.7	-6.0	23.8	X	

GSE32062: Advanced HGS ovarian cancer: HH/PI3K pathway combination

Public dataset Results
- only HGS ovarian cancer samples: PFS

- GSE9891 *Tothill et al. Cancer Res 2008*

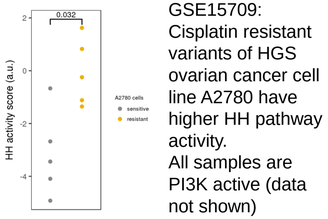


HH pathway activity → bad prognosis

Ovarian cancer cell lines

array	sample	FOXO	HH	NFkB
GSM341221	Carboplatin 24 hours set 1	-1.7	-4.6	3.5
GSM341227	Carboplatin 24 hours set 2	-2.0	-4.8	3.6
GSM341222	Carboplatin 30 hours set 1	-0.2	-5.1	6.8
GSM341228	Carboplatin 30 hours set 2	1.2	-4.6	7.7
GSM341223	Carboplatin 36 hours set 1	0.2	-5.1	8.3
GSM341229	Carboplatin 36 hours set 2	1.4	-6.4	9.0
GSM341224	Vehicle-Control 24 hours set 1	-1.6	-3.2	3.1
GSM341230	Vehicle-Control 24 hours set 2	-4.8	-3.4	0.8
GSM341225	Vehicle-Control 30 hours set 1	-3.4	-4.4	2.0
GSM341231	Vehicle-Control 30 hours set 2	-4.3	-5.2	1.7
GSM341226	Vehicle-Control 36 hours set 1	-1.4	-5.2	2.9
GSM341232	Vehicle-Control 36 hours set 2	-0.2	-4.5	2.5

GSE13525: 36M2 serous ovarian cancer cell line treated with carboplatin (100µM): induction of apoptotic changes, associated with decreased HH/PI3K activity and increased FOXO/NFkB activity

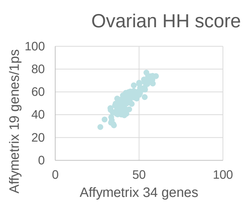


GSE15709: Cisplatin resistant variants of HGS ovarian cancer cell line A2780 have higher HH pathway activity.
All samples are PI3K active (data not shown)

Translation to qPCR for routine diagnostics

The Affymetrix models were ported to an RT-qPCR platform with 96 real-time qPCR assays.

Translation from 34 target genes to 12 genes for qPCR plate



OncoSignal qPCR test procedure and cloud-based pathway activity analysis

Conclusion

Functional signal transduction pathway activity can be quantitatively measured using Philips pathway analysis on Affymetrix microarray and RT-qPCR data. Combined patient studies (including public GEO datasets) and cell line results suggest that HH/PI3K pathway activity in HGS ovarian cancer is associated with bad prognosis and platin resistance.

Summary results

- HGS: two subtypes: HH/PI3K pathway active and FOXO/NFkB pathway active.
- HH activity associated with bad prognosis
- Patient with high HH/PI3K pathway activity: primary platin resistance and rapid progression.
- Ovarian cancer cell line (A2780): HH/PI3K pathway activity associated with platin resistance.
- Activity of NFkB/FOXO may be associated with less aggressive phenotype. In platin-sensitive ovarian cancer cell line, platin induces NFkB/FOXO activity, associated with apoptotic changes.

Discussion

HH activity causing platin resistance has been described for gastric cancer (Yoon et al, Clin Cancer Res. 2014;20(15):3974-88.)
Possible mechanism: HH transcription factor GLI induces expression of the Nucleotide Excision Repair protein XPD, which can repair platin-induced DNA damage.
Signal transduction pathway analysis may provide valuable information to decide on targeted therapy and immune therapy choice.

PHILIPS

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