SEARCH FOR PREDICTIVE BIOMARKERS OF SENSITIVITY/RESISTANCE AGAINST NOVEL FGFR INHIBITOR USING ARRAY-BASED COMPARATIVE GENOMIC HYBRIDIZATION (aCGH) AND RNA SEQUENCING (RNAseq) – PRELIMINARY RESULTS

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INTRODUCTION

Our project concerns characterization of cellular response to CPL-304-110, a novel selective inhibitor of Fibroblast growth factor receptor (FGFR), recently developed by Polish pharmaceutical company CelonPharma. We search for potential biomarkers with the ability to predict response to this drug.

In this preliminary study we analyzed signaling pathways which are differentially regulated in two variants of H1703 lung cancer cell line: wild type, which is sensitive to CPL-304-110 and H1703-R which is resistant.

Affected signalling pathways were selected based on the data from array-based comparative genomic hybridization (aCGH) and from new generation sequencing (NGS) of the transcriptome (RNA-seq).

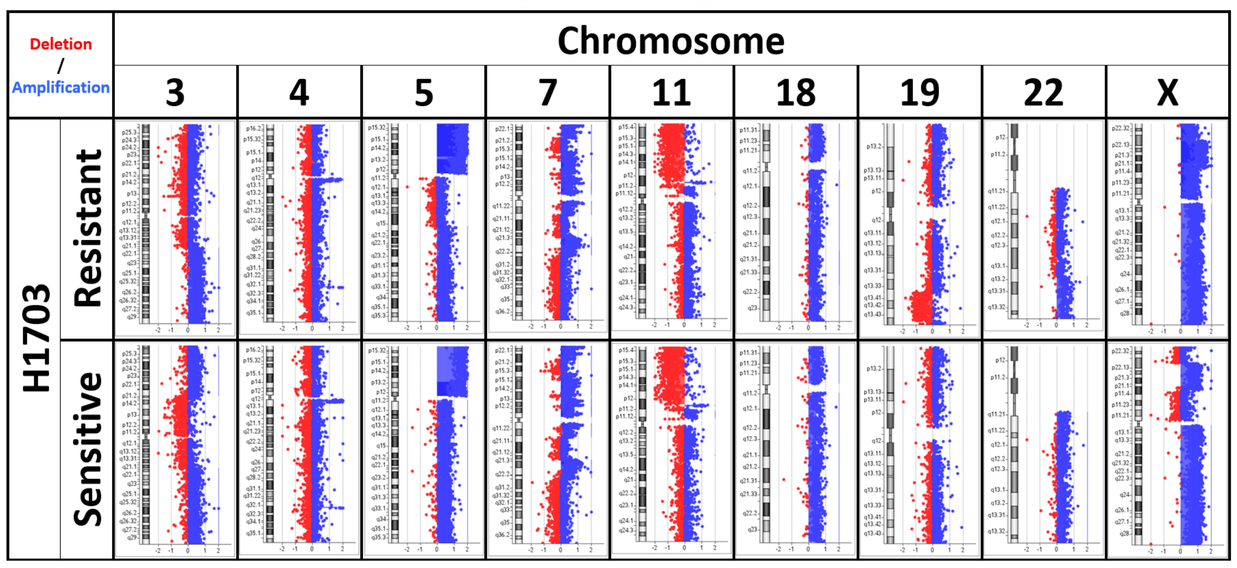
AIM

The aim of this preliminary study was to assess which signaling pathways are differentially regulated in lung cancer cell line H1703-R, resistant to CPL-304-110, as compared to wild type (sensitive) H1703 cells.

Next, we will try to find within these pathways the genes with significantly changed expression. Such genes may potentially be used as a predictive biomarkers correlated with the cancer cell sensitivity or resistance to the novel FGFR inhibitor, CPL-304-110.

RESULTS AND DISCUSSION

Development of resistance towards novel FGFR inhibitor CPL-304-110 induces significant genomic rearrangements



Array-based comparative genomic hybridization (aCGH) revealed significant copy numer changes (amplifications and deletions) in the genome of resistant *versus* sensitive cells.

The Table shows copy numer changes compared between resistant and sensitive H1703 cell lines (only selected chromosomes). Red color denotes deletions while blue – amplifications. Genomic DNA was analyzed by hybridization to the 60K SurePrint G3 Unrestricted CGH arrays (Agilent) and subsequent comparison to the reference genome (normal human Caucassian male).

List of genes which differ by copy number between resistant (R) and sensitive (S) H1703 cell line variants

Increase of copy number							
H1703_S				H1703_R			
No	Gene symbol	No	Gene symbol	No	Gene symbol	No	Gene symbol
1	ORAI2	29	PJA2	1	CAV3	28	TBCA
2	ZNF3	30	TTC	2	CNTN	29	SGTB
3	MAGEB4	31	COG5	3	LSM3	30	PTK2
4	KLHL34	32	NF3	4	PP2D1	31	OC90
5	GLRA2	33	DUS4L	5	NGLY1	32	RNF139
6	PNPLA4	34	SRPK2	6	VIPR1	33	RAD21
7	ADAM3A	35	ANGPT1	7	ADAM3A	34	RSP02
8	FGFR1	36	UBR5	8	FGFR1	35	YWHAZ
9	ZNF703	37	RIDA	9	ZNF703	36	TMEM67
10	LHFPL4	38	FSBP	10	SCAP	37	CA2
11	CNTN4AS2	39	CALB1	11	LINC00971	38	TEK
12	LSG1	40	CA13	12	FAM86DP	39	TSPAN18
13	HRG	41	LOC10124	13	EOGT	40	DSC3
14	PIK3CA	42	NF837	14	ASB14	41	MRO
15	TERC	43	MIR522	15	LSG1	42	EPG5
16	MMDJ	44	CARD8	16	HRG	43	TCF20
17	DHX36	45	SBF1	17	TERC	44	CBX6
18	PLOD2	46	RIBC2	18	NMD3	45	MCM5
19	NCK1	47	PARVB	19	DHX36	46	SFI1
20	TRH	48	RNU12	20	NCK1	47	TTC28
21	LOC33997	49	TEF	21	TLX3	48	CRKL
22	SNCB	50	SLC25A6	22	IL12B	49	MIR3915
23	FBLL1	51	SPIN3	23	C5orf43	50	MIR6134
24	TIMD4	52	GSPT2	24	IL4	51	POLA1
25	SPINK1	53	KDM6A	25	SNX2	52	YY2
26	KLHL3	54	DYNLT3	26	PJA2	53	CDKL5
27	ISCC1	55	SLC25A6	27	POU5F2	54	PIR
28	DMXL1						

Decrease of copy number				
H1703_S		H1703_R		
No	Gene symbol	No	Gene symbol	No	Gene symbol
1	DPYD	1	VILL	26	CCL26
2	IVLNBP	2	CADM2	27	TK1
3	FHIT	3	F3IP1	28	DCC
4	FOXP1	4	FAS	29	MIAT
5	MIR1284	5	BCR	30	MEI1
6	CASC6	6	LY9	31	POU3F4
7	PERP	7	GLB1	32	NRK
8	ZNF8	8	VGLL3		
9	PIK3CG	9	ROBO1		
10	IDE	10	CNTN3		
11	LOC646813	11	MITF		
12	LOC441601	12	MAGI1		
13	DLG2	13	ERC2		
14	MED1	14	MFN1		
15	FUZ	15	LIPH		
16	BAX	16	OPA1		
17	ACPT	17	CNOT6		
18	HAS1	18	MRPL1		
19	ZNF28	19	FRAS1		
20	MIR935	20	KIF2A		
21	FCAR	21	BTF3		
22	NLRP8	22	FTMT		
23	ZIM2	23	MLN		
24	MACROD2	24	HNRNPI		
25	TCF20	25	LOC440040		

Legend
+1 gene copy
+2 gene copy
-1 gene copy
-2 gene copy

The copy number of particular gene was assessed based on comparison with reference genome (normal human Caucassian male).

Genes up- and downregulated in resistant H1703 *versus* sensitive H1703 (RNA-seq results)

UPREGULATED GENES			
Gene Symbol	Gene name	Function	FC
NR0B1	nuclear receptor subfamily 0 group B member 1	DNA-binding transcription factor activity	44,95
COL1A1	Collagen alpha-1(I) chain	extracellular matrix organization, protein binding	24,32
CXCL3	C-X-C motif chemokine 3	plays a role in inflammation and as a chemoattractant for neutrophils	21,82
GDF15	growth differentiation factor 15	secreted ligand of the TGF-beta superfamily of proteins	16,81
ERBB4	erb-b2 receptor tyrosine kinase 4	induces a variety of cellular responses	14,49
MAT1A	methionine adenosyltransferase 1A	metabolism of amino acids and derivatives	14,43
CXCL1	C-X-C motif chemokine ligand 1	plays a role in inflammation and as a chemoattractant for neutrophils	11,75
APOE	apolipoprotein E	is essential for the normal catabolism of triglyceride-rich lipoprotein constituents	8,93
LGALS3BP	galectin 3 binding protein	implicated in modulating cell-cell and cell-matrix interactions	7,46
FGFR3	fibroblast growth factor receptor 3	implicated in mitogenesis and differentiation	5,33

DOWNREGULATED GENES			
Gene Symbol	Gene name	Function	FC
MFAP5	microfibril associated protein 5	extracellular matrix organization	< 0,01
CDH4	cadherin 4	adherens junction organization	< 0,01
SOX2	SRY-box 2	transcription factor involved in the regulation of embryonic development and in the determination of cell fate	0,01
SERPINB7	serpin family B member 7	function as protease inhibitor	0,02
SPRY1	sprouty RTK signaling antagonist 1	negative regulation of MAP kinase activity	0,02
FLRT2	fibronectin leucine rich transmembrane protein 2	chemorepellent activity	0,05
SFTA1P	surfactant associated 1, pseudogene	pseudogene-derived long noncoding RNA SFTA1P exerts the tumor suppressor functions in human lung adenocarcinoma	0,16
DUSP9	dual specificity phosphatase 9	negatively regulates members of the MAP kinase superfamily	0,17
DUSP5	dual specificity phosphatase 5	negatively regulates members of the MAP kinase superfamily	0,26
CDKN1A	cyclin dependent kinase inhibitor 1A	functions as a regulator of cell cycle progression at G1	0,49

FC - fold change

The Tables show 10 upregulated (red) and 10 downregulated (green) genes in resistant H1703-R *versus* sensitive H1703 cells (Arbitrarily selected from among top most highly changed transcripts and showing the highest statistical significance). Transcriptome sequencing (RNA-seq) was performed on the Illumina HiSeq4000 Platform using the standard paired-end protocol.

Signaling pathways highly affected in H1703 cell line due to development of resistance to FGFR inhibitor

H1703_R

aCGH

Pathway name	Entities		
	found	pValue	FDR
FGFR1 mutant receptor activation ★	4/44	0,000202	0,0413
Signaling by FGFR1 in disease ★	4/53	0,000407	0,0413
PI3K events in ERBB2 signaling	3/22	0,000413	0,0413
Constitutive Signaling by Aberrant PI3K in Cancer	5/99	0,000475	0,0413
GRB7 events in ERBB2 signaling	2/6	0,00072	0,048
PI-3K cascade:FGFR1 ★	3/28	0,000828	0,048
PI5P, PP2A and IER3 Regulate PI3K/AKT Signaling	5/122	0,0012	0,0543
Negative regulation of the PI3K/AKT network	5/130	0,00159	0,0543
PI3K/AKT Signaling in Cancer	5/130	0,00159	0,0543
Signaling by FGFR1 amplification mutants ★	2/9	0,0016	0,0543
Downstream signaling of activated FGFR1 ★	3/41	0,00245	0,0759
Signaling by FGFR in disease ★	4/89	0,00271	0,0787
PIP3 activates AKT signaling	7/312	0,0041	0,0923
Signaling by activated point mutants of FGFR1 ★	2/15	0,00433	0,0923
PI3K events in ERBB4 signaling	2/15	0,00433	0,0923
Erythropoietin activates Phosphoinositide-3-kinase (PI3K)	2/16	0,0049	0,0923
Downregulation of ERBB2:ERBB3 signaling	2/16	0,0049	0,0923
ERBB2 Activates PTK6 Signaling	2/18	0,00616	0,0923
PI3K Cascade	3/58	0,00641	0,0923
ERBB2 Regulates Cell Motility	2/19	0,00683	0,0923
Signaling by FGFR1 ★	3/61	0,00735	0,0923
Signaling by ERBB4	3/61	0,00735	0,0923
Intracellular signaling by second messengers	7/351	0,00766	0,0923
FGFR1 ligand binding and activation ★	2/21	0,00827	0,0923
IRS-mediated signalling	3/64	0,00838	0,0923
RAF/MAP kinase cascade	6/273	0,00855	0,0923
Phospholipase C-mediated cascade: FGFR1 ★	2/22	0,00904	0,0923
Tie2 Signaling	2/22	0,00904	0,0923
Signaling by ERBB2	3/67	0,00948	0,0923
MAPK1/MAPK3 signaling	6/280	0,00961	0,0923

RNAseq

Pathway name	Entities		
	found	pValue	FDR
Collagen biosynthesis and modifying enzymes	8/76	0,000211	0,0672
Degradation of the extracellular matrix	11/148	0,000301	0,0672
Collagen formation	9/104	0,000361	0,0672
Activation of Matrix Metalloproteinases	5/35	0,000872	0,129
Downregulation of ERBB4 signaling	3/10	0,00124	0,153
Collagen chain trimerization	5/44	0,00236	0,25
Assembly of collagen fibrils and other multimeric structures	6/67	0,00289	0,255
Scavenging by Class A Receptors	5/48	0,00341	0,255
Extracellular matrix organization	15/329	0,00383	0,255
Anchoring fibril formation	3/15	0,00387	0,255
Laminin interactions	4/31	0,00412	0,255
Signaling by activated point mutants of FGFR3 ★	3/17	0,00547	0,312
ERBB2 Activates PTK6 Signaling	3/18	0,0064	0,314
ERBB2 Regulates Cell Motility	3/19	0,00741	0,341
SHC1 events in ERBB4 signaling	3/20	0,00852	0,348
GRB2 events in ERBB2 signaling	3/20	0,00852	0,348
Non-integrin membrane-ECM interactions	5/61	0,00914	0,348
Glycosphingolipid metabolism	6/86	0,0094	0,348
Crosslinking of collagen fibrils	3/24	0,0139	0,461
Regulation of PTEN gene transcription	5/70	0,0157	0,461
FGFR3 mutant receptor activation ★	3/26	0,0171	0,461
Other interleukin signaling	3/26	0,0171	0,461
Negative regulation of activity of TFAP2 (AP-2) family transcription factors	2/11	0,022	0,461
Transcriptional regulation by the AP-2 (TFAP2) family of transcription factors	4/52	0,0234	0,461
ECM proteoglycans	5/79	0,0248	0,461
Nuclear Receptor transcription pathway	5/84	0,0311	0,461
Integrin cell surface interactions	5/86	0,0339	0,461
RUNX2 regulates osteoblast differentiation	3/34	0,034	0,461
SHC1 events in ERBB2 signaling	3/35	0,0365	0,461
Adherens junctions interactions	3/35	0,0365	0,461

★ **Signaling pathways involving FGFR**

Signaling Pathways concordantly revealed by aCGH and RNAseq

Signaling pathways were selected using REACTOME Pathway Knowledgebase, based on the list of genes obtained from aCGH and RNAseq experiments.

CONCLUSIONS

- **Our study allowed to detect genome rearrangements and gene expression changes in H1703 lung cancer cells, which were induced during development of resistance toward the novel FGFR inhibitor CPL-304-110 (CelonPharma).**
- **The results of aCGH indicate that genomic regions affected by copy numer changes (amplifications and deletions) contribute to signaling pathways involved in proliferation, survival, apoptosis, differentiation, cell cycle, cellular motility and metabolism.**
- **Sequencing of cellular transcriptome (RNAseq) revealed that resistant cell line H1703-R shows significantly changed expression of many genes engaged in signaling pathways related with structure and function of extracellular matrix. Interestingly, among affected pathways were also those related with FGFR signaling, ERBB and GRB2 signaling, regulation of PTEN gene transcription.**
- **Further studies are in progress that will select and validate potential molecular markers of sensitivity/resistance toward novel FGFR inhibitor. Such molecular markers will help to identifiy the subset of patients that can benefit from the therapy with this novel drug.**

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