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Cell-based Screening Identifies Gene Expression Signature Correlated with Sensitivity to MEK Inhibitor Trametinib

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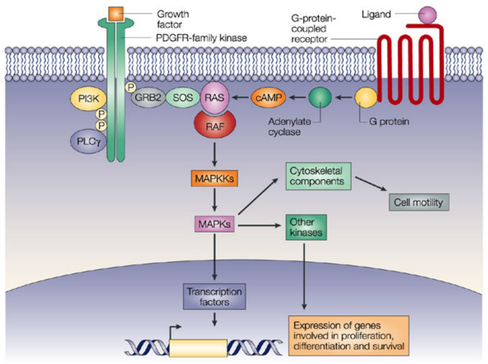
Introduction

The Ras-Raf-MEK-ERK signaling cascade is one of the most well known pro-proliferation and pro-survival pathways involved in tumor growth and progression. Activation of RAS GTPases recruits Raf to the cell membrane which sequentially phosphorylates and activates serine/ therionine kinases MEK and ERK. Ras and Raf mutations are frequently detected in many types of human cancers, leading to over-activation MEK and ERK kinase and their downstream effector molecules.



Nature Reviews | Cancer

Abstract

Multiple MEK inhibitors are currently under clinical investigation for cancer treatment and several agents targeting MEK are under preclinical development. The mechanisms underlying resistance to MEK inhibitor are unclear. Here we examined the anti-proliferation activity of a MEK inhibitor, trametinib, in a panel of cell lines harboring either Ras or Raf gene mutation. We found 34 lines that are sensitive to the inhibitor and 24 lines are resistant. We compared gene expression by Affymetrix U219 arrays and gene copy number by Affymetrix SNP6.0 arrays in these two cell line groups. With a p-value cutoff set at 0.01, we identified 549 genes differentially expressed and 328 genes demonstrated different copy numbers between the two groups. 23 genes were both detected by gene expression profiling and gene copy number data. These genes include a list of important molecule markers in cancer biology, including FOXO1 and JAG1. Furthermore, the study identified 3 genes whose mutation status strongly correlated with sensitivity to trametinib. Collectively, these data suggested a potential genomic signature that could be predictive of response to the MEK inhibitor *in vitro*. Further investigation and validation in patient-derived xenograft (PDX) models will undoubtfully provide valuable information for selecting patients that are likely to receive clinical benefit.

Methods

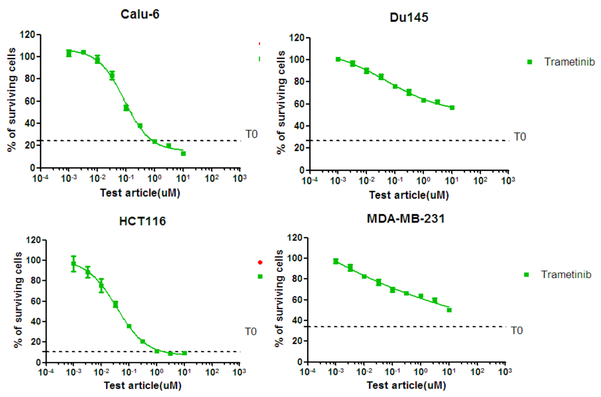
We used Crown Ras/raf mutation panel of 51 cell lines and 12 lines without the mutations as a control panel. We performed cell viability assay to determined IC50s of trametinib in the 63 cell lines. Based on IC50 values, we divided the cell lines to 3 groups: sensitive, insensitive and intermediate. 34 cell lines are sensitive to trametinib and 24 are insensitive. We then performed gene expression and pathway enrichment analysis using GSEA software.

Cell Lines

Cell lines	Ras/raf mutation	Cell lines	Ras/raf mutation	Cell lines	Ras/raf mutation
A549	Yes	PANC-1	Yes	CFRAC-1	Yes
A2058	Yes	Sk-Hep-1	Yes	Du4475	Yes
Calu6	Yes	Sk-Mel-5	Yes	HuCCT1	Yes
DLD-1	Yes	BT474	Yes	NCI-H1395	Yes
HCT116	Yes	Colo205	Yes	NCI-H1573	Yes
HepG2	Yes	KM12L4	Yes	SNU-719	Yes
MDA-MB-231	Yes	MIAPaCa2	Yes	SNU-387	Yes
NCI-H23	Yes	NCI-H1155	Yes	SW1116	Yes
NCI-H460	Yes	NCI-H1373	Yes	SW1271	Yes
RKO	Yes	NCI-H1651	Yes	22RV1	No
SW620	Yes	NCI-H1666	Yes	BxPc-3	No
SW480	Yes	NCI-H2009	Yes	HCC2935	No
HCT-8	Yes	NCI-H2227	Yes	HCC4006	No
HCT-15	Yes	PL45	Yes	Hela	No
HT29	Yes	SK-LU-1	Yes	Hep3B	No
LoVo	Yes	SNU-1	Yes	HM-7	No
LS513	Yes	ZR-75-1	Yes	HT1376	No
NCI-H358	Yes	A375	Yes	KYSE150	No
NCI-H441	Yes	AGS	Yes	NCI-H1703	No
NCI-H1299	Yes	ASPC-1	Yes	PC-3	No
NCI-H1792	Yes	Capan-1	Yes	Du145	No

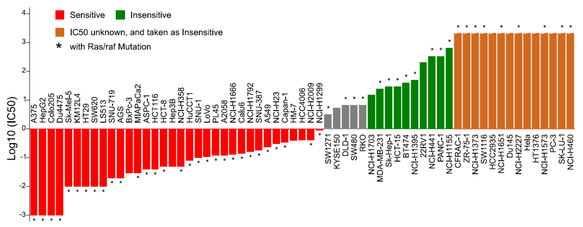
Results

Representative trametinib-sensitive and resistant cell lines



Results

Crown Ras/Raf mutation cell panel screen for trametinib identified sensitive and resistant cell lines

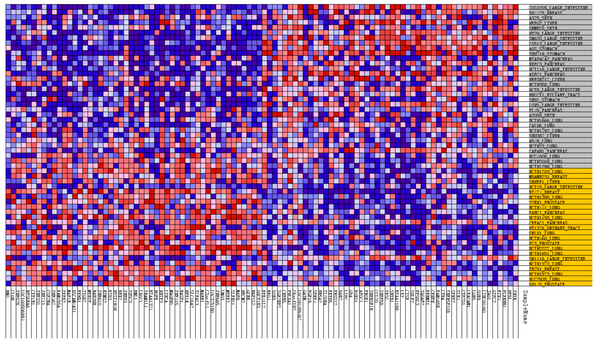


Crown Ras/Raf mutation cell panel screen for trametinib identified sensitive and resistant cell lines

Cell line	IC50 (uM)	Max inhibition (%)	Cell line	IC50 (uM)	Max inhibition (%)	Cell line	IC50 (uM)	Max inhibition (%)
A549	0.24	69.1	PANC-1	330.05	30.1	CFRAC-1	>100	49
A2058	0.12	52.2	Sk-Hep-1	29.14	53	Du4475	0	99.4
Calu6	0.14	87.5	Sk-Mel-5	0.01	59.6	HuCCT1	0.08	68
DLD-1	6.52	56.5	BT474	39	18.6	NCI-H1395	48.85	45.2
HCT116	0.04	91.6	Colo205	0	84.9	NCI-H1573	>100	51
HepG2	0	87.9	KM12L4	0.01	80.4	SNU-719	0.02	93.9
MDA-MB-231	23.98	50.8	MIAPaCa2	0.03	69.6	SNU-387	0.18	82.4
NCI-H23	0.3	63.3	NCI-H1155	636.34	27.6	SW1116	>100	46.4
NCI-H460	>100	51.9	NCI-H1373	>100	42.9	SW1271	3.15	61.1
RKO	6.7	58.8	NCI-H1651	>100	24.9	22RV1	202.02	50.9
SW620	0.01	67.1	NCI-H1666	0.13	75	BxPc-3	0.03	74.4
SW480	6.65	53.5	NCI-H2009	0.41	67.8	HCC2935	>100	40.7
HCT-8	0.05	74.7	NCI-H2227	>100	13.6	HCC4006	0.41	78.2
HCT-15	29.33	50.5	PL45	0.12	65.8	Hela	>100	48
HT29	0.01	79	SK-LU-1	>100	45.4	Hep3B	0.05	93.3
LoVo	0.11	76.8	SNU-1	0.1	73.5	HM-7	0.39	58.1
LS513	0.01	97.3	ZR-75-1	>100	15.2	HT1376	>100	50.3
NCI-H358	0.05	74.5	A375	0	77.2	KYSE150	5.28	58.5
NCI-H441	324.66	30.4	AGS	0.02	97.2	NCI-H1703	14.99	47.7
NCI-H1299	0.91	59.9	ASPC-1	0.04	69.1	PC-3	>100	6.8
NCI-H1792	0.16	90.4	Capan-1	0.34	64	Du145	>100	46.2

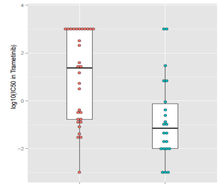
Bioinformatic Analysis

Top 50 genes (528 total) whose expression levels are significantly different in sensitive and resistant cell lines



Bioinformatic Analysis

Bioinformatic analysis reveals 3 genes whose mutation status strongly correlate with sensitivity to trametinib.



Cpd	gene	sensitive with mutation	sensitive without mutation	insensitive with mutation	insensitive without mutation	p-value
Trametinib	gene 1	0	29	5	15	0.0081305
Trametinib	gene 2	0	29	5	15	0.0081305
Trametinib	gene 3	12	17	17	3	0.0030596

Gene that are different expressed in trametinib sensitive and resistant are enriched in 34 known cancer signaling pathways

Pathway Name	Number of genes	Normalized p-value
REACTOME_FRS2_MEDIATED_CASCADE	36	0
BIOCARTA_FCER1_PATHWAY	38	0
KEGG_THYROID_CANCER	29	0
BIOCARTA_HCMV_PATHWAY	17	0
REACTOME_NEGATIVE_REGULATION_OF_FGFR	36	0
PID_CMYB_PATHWAY	83	0
REACTOME_SIGNALING_BY_FGFR	108	0.001876173
REACTOME_DOWNSTREAM_SIGNALING_OF_FGFR	97	0.001886793
PID_ECADHERIN_KERATINOCYTE_PATHWAY	19	0.001937985
BIOCARTA_SPPA_PATHWAY	19	0.001984127
PID_FOXM1PATHWAY	38	0.003787879
PID_RAC1_PATHWAY	52	0.003838772
BIOCARTA_BARRESTIN_SRC_PATHWAY	15	0.003868472
REACTOME_DOWNREGULATION_OF_SMAD2_3_SMAD4	19	0.003883495
REACTOME_SIGNALING_BY_FGFR_IN_DISEASE	121	0.003883495
REACTOME_ANTIGEN_ACTIVATES_B_CELL_RECEPTOR	29	0.00390625
REACTOME_SHC_MEDIATED_CASCADE	28	0.003913894
PID_DNAPK_PATHWAY	15	0.004040404
REACTOME_PI_3K_CASCADE	54	0.005576208
BIOCARTA_PDGF_PATHWAY	32	0.005747126
BIOCARTA_PYK2_PATHWAY	27	0.00589391
REACTOME_SIGNALING_BY_TGF_BETA_	60	0.005905512
PID_PDGFRBPATHWAY	126	0.006097561
BIOCARTA_ECM_PATHWAY	22	0.006302521
BIOCARTA_AT1R_PATHWAY	31	0.00742115
BIOCARTA_FMLP_PATHWAY	35	0.007736944
BIOCARTA_IGF1_PATHWAY	21	0.007751938
PID_RB_1PATHWAY	64	0.007782101
PID_IL3_PATHWAY	27	0.007858546
REACTOME_DEADENYLATION_OF_MRNA	15	0.008032128
PID_NOTCH_PATHWAY	58	0.008064516
REACTOME_TRANSCRIPTIONAL_ACTIVITY_OF_SMAD2_SMAD3_SMAD4_HETEROTRIMER	36	0.009596929
REACTOME_FGFR_LIGAND_BINDING_AND_ACTIVATION	22	0.009861933
PID_CXCR4_PATHWAY	99	0.009861933

Conclusions

- Using Crown Oncogene-specific panel with Ras/Raf mutation, we have identified 528 genes whose expression levels significantly correlate with trametinib sensitivity.
- Mutation status of 3 cancer-associated genes strongly correlates with trametinib sensitivity.
- The results proposed genetic signatures that predict sensitivity to trametinib. Further investigation on these gene will provide valuable information for clinical trials as well as personalized medicine.

Contact us at www.crownbio.com for detailed information on cell panels