

Genome-wide Analysis Suggests that cMET is a Driver of RAS Signaling and Implicates RAS Activation Status as a Predictor for Response to cMET Inhibitors

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Abstract

- In order to facilitate the development of HGF/ c-MET inhibitors, we undertook a genome-wide analysis to identify a "c-MET activation signature" that could be used to identify tumors that are potentially dependent on HGF/ c-MET signaling.
- Our previously reported RAS signature (Loboda et al., 2009 AACR annual meeting) was the top pathway enrichment, indicating a link between genes involved in RAS signaling and cMET expression across tumors. A strong correlation between c-MET expression and our RAS signature was present in multiple tumor types and was conserved in metastatic tumors.
- Using reverse-phase protein arrays measuring approximately 70 proteins across a panel of 89 lung cancer cell lines, we found that pMEK, pERK, and pMET were correlated with our RAS signature. Taken together, these data suggest that cMET can serve as a driver of RAS signaling.
- To test this, we performed a xenograft study in which GTL-16 xenografts (MET amplified gastric cancer cell line) were treated with a novel small molecule inhibitor of c-MET (MK-8033). MK-8033 treatment caused a dose- and time-dependent inhibition of the RAS signature at all three time points, and the level of RAS signature inhibition was related to efficacy.
- In Ovarian cancer cell lines, RTK-arrays showed high phospho-cMET (4/8 lines) and co-activation of EGFR and Her2/4. Moreover, HGF was shown to activate the cMET/RAS pathway, and MK-8033 had anti-cancer efficacy in soft agar colony formation assays.
- These data support the hypothesis that cMET is a driver of RAS signaling in some tumor contexts and suggest that basal levels of RAS pathway activation may be an important determinant of response to HGF/ c-MET inhibition in the clinic.

Derivation of a cMET co-expression signature

We started by identifying a "cMET co-expression" signature by identifying genes that correlate with expression of cMET across internal tumor profiling databases. In addition to other genes, EGFR was one of the top hits, indicating that EGFR and cMET are often co-expressed in tumors

Genes correlating with cMET expression across tumors

	All rho	All p-value	Lung rho	Lung p-value	Colon rho	Colon p-value
MEY	98%	0E+00	98%	0E+00	97%	0E+00
MEY	97%	0E+00	97%	0E+00	93%	0E+00
EPHA2	64%	1E-193	33%	1E-08	38%	3E-10
EGFR	64%	7E-192	42%	2E-14	39%	7E-09
TGFA	64%	2E-191	42%	8E-14	39%	8E-11
HKDC1	63%	2E-179	36%	4E-10	49%	6E-17

Pathway enrichment analysis indicated that gene expression signatures of RAS pathway activity are most significantly enriched within the cMET co-expression signature

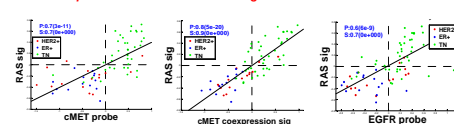
RAS pathway signatures highly correlate with c-MET expression across tumor types

	All rho	All p-value	Brain rho	Brain p-value	Lung rho	Lung p-value	Colon rho	Colon p-value
Merck RAS Signature	59%	7E-173	57%	0E+00	50%	0E+00	35%	0E-04
Bid et al RAS signature, Nature 439, 353-357	59%	2E-168	56%	0E+00	50%	0E+00	36%	0E-04
Genes regulated by MEK1, internal	46%	1E-95	42%	5E-07	47%	0E+00	24%	3E-02

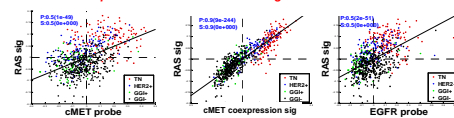
cMET correlates with the Merck RAS signature in cell lines and across tumor types

To further assess this relationship, we assessed the correlation between the cMET probe (i.e. the microarray probe that measures the level of expression of cMET), the EGFR probe, the cMET co-expression signature, and our RAS signature across breast cancer cell lines and tumors

cMET expression correlates with RAS signature across breast cancer cell lines

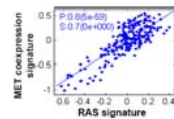


cMET expression correlates with RAS signature across breast tumors



Met expression is highest in triple negative breast cancer, cMET could be driving RAS signaling in the absence of Kras mutations in breast cancer

cMET expression correlates with RAS signature across lung tumors

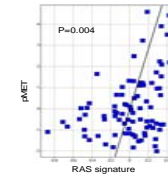


Correlation preserved in lung cancer, cMET could be driving RAS signaling in the absence of Kras mutations in breast cancer

Phospho-MET correlates with the Merck RAS signature across lung cancer cell lines

The relationship between c-MET and RAS activation was assessed using reverse-phase protein arrays (70 proteins across a panel of 89 lung cancer cell lines). Four proteins were identified that significantly correlated positively with the RAS signature: total ERBB4, pMEK, pERK, and pMET

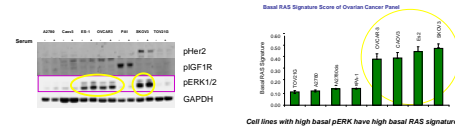
RPPA node	Pearson p-value
ERBB4	0.0005059
pMEK	0.0005957
pMET	0.004356
pERK	0.006344



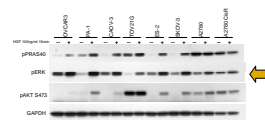
Hypothesis: cMET activity is a driver of RAS signaling

HGF treatment induces pERK in cell lines

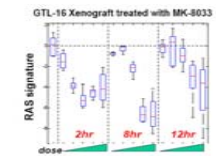
Western blot analysis of Ovarian cancer cell lines either serum starved or stimulated



Western blot analysis of Ovarian cancer cell lines stimulated with HGF



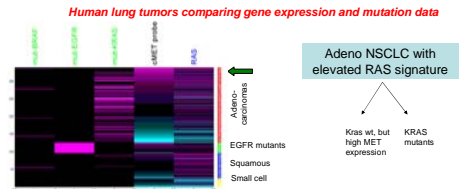
cMET inhibition down-regulates the Merck RAS signature in an in vivo, cMET-driven xenograft model



MET amplified gastric cancer xenograft

GTL16 xenograft animals were treated with vehicle or with a single dose of 10, 33, 66, 100, or 200 mg/kg MK-8033. At each dose, we collected samples for profiling at 2, 8, and 12 hours post-dose. The figure shows the impact of MK-8033 on the RAS signature compared to time-matched vehicle. 0 = no change, a negative number indicates downregulation. MK-8033 inhibited the RAS signature in a dose- and time-dependent manner.

K-ras wt, high RAS signature NSCLC adenocarcinomas could be driven by c-MET expression



Conclusions

- A novel c-MET co-expression signature is highly enriched for previously reported RAS signatures.
- cMET expression correlates with a RAS pathway signatures across multiple tumor types.
- pMET protein also correlates with our RAS pathway signature.
- HGF treatment induces RAS signaling components like pERK.
- cMET inhibition down regulates our RAS pathway signature.
- Kras wild-type lung tumors that have high RAS signature scores express high levels of cMET.
- cMET is a driver of RAS signaling, and tumors expressing elevated RAS signals in the absence of K-ras mutations may be a responder population for cMET inhibitors.