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In vitro tyrosine kinase activity profiling to identify molecular targets and predictive biomarkers in gastric cancer cell lines

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Abstract

Background: Gastric cancer is among the leading causes of cancer-related deaths worldwide, notably in Asia. Though targeted therapies have been under clinical evaluation, the biological complexity of this cancer type may confer resistance to single-targeted therapies. Especially, scirrhous phenotype is known to be affected by multiple growth factors. To overcome this biological complexity, multi-targeted kinase inhibitors (MTKIs) have been clinically tested and were reported to be effective in a subset of patients. We wanted to elucidate the kinase activity signature of gastric cancer and to explore predictive biomarkers of MTKIs using gastric cancer cell lines by tyrosine kinase activity profiling.

Methods: The growth-inhibitory effect of the MTKIs sunitinib, sorafenib, pazopanib and the Akt inhibitor MK-2206 was evaluated by the MTT assay. 11 scirrhous and 14 non-scirrhous gastric cancer cell lines were grown until semi-confluent, lysed, aliquoted and stored at -80°C until use. The basal tyrosine kinase activity of the lysates was measured on PamChip® peptide micro-arrays, containing 144 peptides derived from known human phosphorylation sites. Quantification of peptide phosphorylation and data analysis were performed using BioNavigator and SIMCA P+ software.

Results: HSC-39, HSC-40A, KATO-III and HSC-43 scirrhous cells and SNU-16 non-scirrhous cells were sensitive to all three MTKIs with IC50 values < 1 μmol/L. Orthogonal partial least square-discriminant analysis (OPLS-DA) of basal tyrosine kinase activity profiles showed a clear segregation between sensitive cell lines and insensitive cell lines. Levels of putative target proteins such as PDGFR, VEGFR, Raf and Kit did not correlate with the sensitivity. All sensitive cell lines turned out to harbor an FGFR2 amplification suggesting that FGFR2 amplification may be a predictive biomarker of response to MTKI treatment. Kinases such as EGFR, EPHA, FGFR, IGF1R, INSR and Src contributed to this segregation suggesting that these are relevant to the sensitivity to MTKIs. MET overexpressing cell lines were resistant to treatment with the four inhibitors. Scirrhous cells were segregated from non-scirrhous cells by OPLS-DA among cell lines with signet-ring cell carcinoma cytology with R2, 0.994; Q2, 0.615. EGFR, ERBB2, 3, 4, EPHAs, FAK, IGF1R, INSR, MAP2Ks, RET and SRC seemed to be responsible for the segregation suggesting that they play an important role in scirrhous gastric cancer biology.

ConclusionsKinase activity profiles reflect the biological complexity of gastric cancer. FGFR2 status correlates with sensitivity to sunitinib, pazopanib and sorafenib, whereas MET status does not. These data imply that tyrosine kinase activity profiling with PamChip® peptide arrays can be utilized to identify molecular targets and predictive biomarkers in gastric cancer and this should be further explored.

Dynamic peptide micro-array technology

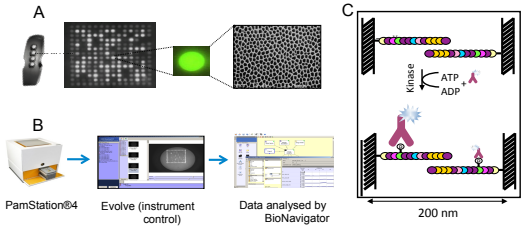


Figure 1. (A)(B) PamStation4 instrument and PamChip4 array plates used for kinase activity profiling. (C) The reaction takes place on spotted peptides on the array. Peptide phosphorylation through active kinases can be monitored in lysates of cell lines by real time fluorescent readout using a fluorescent labeled phospho-tyrosine antibody.

Cell lines

Name	Scirrhous/Non-scirrhous	Histology	Mutation status	response to MKIs: Sunitinib	Sorafenib	Pazopanib
44As3	Scirrhous	Signet-ring cell carcinoma	KRAS G12V			
SBAs1	Scirrhous	Signet-ring cell carcinoma	MET amp			
HSC-39	Scirrhous	Signet-ring cell carcinoma	FGFR2 amp			
HSC-40A	Scirrhous	Signet-ring cell carcinoma	FGFR2 amp			
HSC-43	Scirrhous	Signet-ring cell carcinoma	FGFR2 amp			
HSC-44PE	Scirrhous	Signet-ring cell carcinoma	KRAS G12V			
HSC-45	Scirrhous	Signet-ring cell carcinoma	KRAS G12V			
HSC-58	Scirrhous	Signet-ring cell carcinoma	MET amp			
HSC-59	Scirrhous	Signet-ring cell carcinoma	PIK3CA E545K			
HSC-60	Scirrhous	Signet-ring cell carcinoma				
KATOIII	Scirrhous	Signet-ring cell carcinoma	FGFR2 amp			
IM95	Non-scirrhous	Moderately-differentiated adnocarcinoma	PIK3CA E542K			
JR-st	Non-scirrhous	Signet-ring cell carcinoma				
MKN1	Non-scirrhous	Adenosquamous cell carcinoma	PIK3CA E545K			
MKN28	Non-scirrhous	Moderately-differentiated adnocarcinoma				
MKN45	Non-scirrhous	Poorly-differentiated adnocarcinoma	MET amp			
MKN7	Non-scirrhous	Well-differentiated adnocarcinoma				
MKN74	Non-scirrhous	Moderately-differentiated adnocarcinoma				
NCI-N87	Non-scirrhous	Well-differentiated adnocarcinoma				
NUGC-3	Non-scirrhous	Poorly + signet ring component				
NUGC-4	Non-scirrhous	Poorly + signet ring component				
OCUM-1	Non-scirrhous	Signet-ring cell carcinoma	MEK1 Q56P			
OKAJIMA	Non-scirrhous	Poorly-differentiated adnocarcinoma	MET amp			
SNU-1	Non-scirrhous	Poorly-differentiated adnocarcinoma	KRAS G12D			
SNU-16	Non-scirrhous	Poorly-differentiated adnocarcinoma	FGFR2 amp			

Table 1. Results of mutational testing and growth-inhibitory effect of MTKIs (sunitinib, sorafenib, pazopanib).

Sensitive (IC50 values < 1 μmol/L)
Intermediate
Insensitive (IC50 values >10 μmol/L)

Results

Figure 2. Heatmap analysis according to basal tyrosine kinase activity

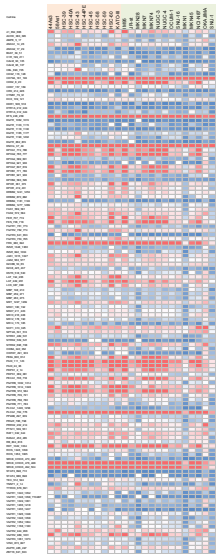


Figure 3. (A) Volcano plot analysis between sensitive cell lines and non-sensitive cell lines to MTKIs. (B) RT-PCR to detect *FGFR2* expression. (C) Copy number analysis on *FGFR2*

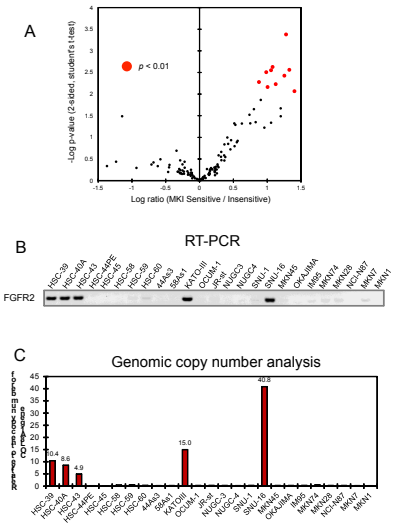


Table 2. Putative kinases relevant to segregate sensitive cell lines

Significant peptides (p<0.01)	Putative kinases contributed to the phosphorylation of peptide
CALM_93_105	ERBB1
	IGF1R
	INSR
DYR1A_212_224	DYR1A
EGFR_1165_1177	ERBB1
	IGF1R
	INSR
EGFR_1190_1202	Abl
	ERBB1
	EPHA3
	EPHA4
	EPHA7
	ERBB2
ERBB2_870_882	IGF1R
	INSR
ERBB4_1181_1193	ERBB4
	IGF1R
	INSR
FGFR1_761_773	EPHA3
	EPHA4
	EPHA7
	FGFR1
FGFR2_762_774	EPHA3
	EPHA4
	EPHA7
	FGFR2
FGFR3_753_765	EPHA3
	EPHA4
	EPHA7
	FGFR3
	IGF1R
	INSR
LAT_194_206	ZAP70
PGFRB_1014_1028	EPHA3
	EPHA4
	EPHA7
	IGF1R
	INSR
	PDGFRB
VGFR2_1168_1180	EPHA3
	EPHA4
	EPHA7
	IGF1R
	INSR
	Src
	VEGFR2

Figure 4. (A) Volcano plot analysis between *MET* amplified cell lines and *MET* non-amplified cell lines. (B) Copy number analysis on *MET*

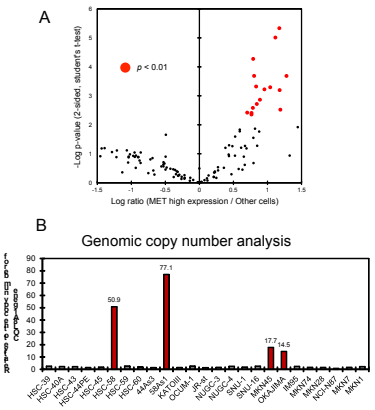
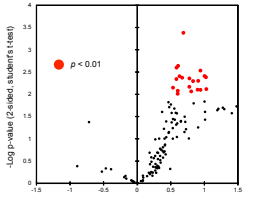


Table 3. Putative kinases relevant to segregate *MET* amplified cell lines

Significant peptides (p<0.01)	Putative kinases contributed to the phosphorylation of peptide
EPHA4_689_601	EPHA3
	EphA4
	EPHA7
EPHB1_921_933	EPHB1
	BTK
	EPHA3
	EPHA4
	EPHA5
MBP_198_210	EPHA7
	IGF1R
	INSR
	ITK
	MET
	TEC
MK14_173_185	ASK1
	MAP2K6_MAP2K3_MAP2K4_MAP2K7_group
	MEKK6
	RET
ODPAT_291_303	IGF1R
	INSR
PTN11_539_551	ALK
	Fyn
	PDGFR

Significant peptides (p<0.01)	Putative kinases contributed to the phosphorylation of peptide
RB_804_816	Abl
	IGF1R
	INSR
STAT4_686_698	CSF1R
	ERBB1
	ERBB2
	ERBB3
	ERBB4
	JAK_group
	KIT
	MAP2K6_MAP2K3_MAP2K4_MAP2K7_group
	PDGFRA
	PDGFRB
VGFR1_1049_1061	IGF1R
	INSR
	VGFR1
VGFR1_1206_1218	FLT1
	IGF1R
	INSR
	VEGFR1
VGFR2_944_956	IGF1R
	INSR
	VEGFR2
VINC_815_827	CSF1R
	KIT

Figure 5. Volcano plot analysis between scirrhous cell lines and non-scirrhous cell lines.



Significant peptides (p<0.01)	Putative kinases contributed to the phosphorylation of peptide
INSR_992_1004	ABL
	BTK
	ERBB1
	ERBB2
	ERBB3
	ERBB4
	INSR
	ITK
	MAP2K6_MAP2K3_MAP2K4_MAP2K7_group
	TEC
KSYK_518_530	IGF1R
	INSR
	KSYK
MET_1227_1239	Met
	Ron
MK01_180_192	EPHA3
	EPHA4
	EPHA7
	JAK2
	MEK1
VGFR2_1052_1064	EPHA3
	EPHA4
	EPHA7
	IGF1R
	INSR
	VEGFR2
VGFR3_1061_1073	EPHA3
	EPHA4
	EPHA7
	IGF1R
	INSR
	SRC
	VGFR3

Table 4. Putative kinases relevant to segregate scirrhous cell lines

Significant peptides (p<0.01)	Putative kinases contributed to the phosphorylation of peptide
ANXA1_14_26	Abl
	ERBB1
	ERBB2
	ERBB3
	ERBB4
	IGF1R
	INSR
	PDGFRA
	PDGFRB
	Src
EGFR_1165_1177	ERBB1
	IGF1R
	INSR
EGFR_1190_1202	Abl
	ERBB1
	EPHA3
	EPHA4
	EPHA7
EPHB4_583_595	EPHA3
	EPHA4
	EPHA7
	IGF1R
	INSR
ERBB2_870_882	ERBB2
	IGF1R
	INSR
ERBB4_1277_1289	ERBB4
	IGF1R
	INSR
FAK1_569_581	FAK
	Fgr
	IGF1R
	INSR
	Met
	Src
FAK2_572_584	FAK2
	IGF1R
	INSR
	PTK2B
FGFR1_761_773	EPHA3
	EPHA4
	EPHA7
	FGFR1
FGFR2_762_774	EPHA3
	EPHA4
	EPHA7
	FGFR2
FGFR3_753_765	EPHA3
	EPHA4
	EPHA7
	FGFR3
	IGF1R
	INSR

Conclusions

- Kinase activity profiles reflect the biological complexity of gastric cancer.
- FGFR2 status seems to correlate with sensitivity to sunitinib, pazopanib and sorafenib.
- Tyrosine kinase activity profiling with PamChip® peptide arrays can be utilized to identify molecular targets.
- Pathway analysis needs to be performed to further identify the relevant targets and predictive biomarkers.