Response prediction to a multitargeted tyrosine kinase inhibitor by profiling serine/threonine kinase activity and inhibition.

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Abstract

Since treatment in oncology is rarely effective in all patients, stratification of patients with innate resistance or monitoring the emergence of acquired resistance will prevent patients being treated with ineffective drugs and will further the development of personal medicine. Several examples of such prediction methods have been reported, based on gene expression or presence of marker proteins. For kinase inhibitors, that target active kinases, it makes sense to monitor their effect on kinase activity ex vivo on a patients own tumor tissue. For the prediction of cell line response to a multityrosine kinase inhibitor (MTKI) in a proliferation assay, the feasibility of this approach has been shown, using tyrosine kinase activity profiling on a dynamic peptide microarray in the presence and absence of this inhibitor. Here, we predict the response to MTKI (i.e. the response in a proliferation assay) by profiling the serine/threonine kinase activities on a peptide microarray.

Methods

24 Cancer cell lines were cultured in the presence of a multi-tyrosine kinase inhibitor (MTKI) and the IC50 was determined in a proliferation assay [1].
Lysates from untreated cells were incubated in triplicate on PamChip® 96 array plates composed of 96 peptide microarrays comprising 140 ser/thr containing peptides on each array. Lysates were incubated in the presence and absence of hydroxystaurosporin, flavopiridol or AZD6244. Peptide phosphorylation by the lysates was monitored using a mixture of phosphoserine and phosphothreonine detecting antibodies. Signal intensities on each peptide spot on each array were quantitated. Ratio's of inhibited/non-inhibited signal intensities were calculated for each peptide and correlated to the proliferation assay data. A peptide subset that gave ATP dependent signals ($p<0.001$ when tested against incubations without ATP) was used for further analysis.

PamChip® Cell-Based Kinase Assay

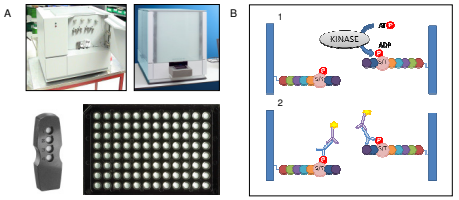

Fig. 1. A. PamStation®4, 12 and 96 instruments and PamChip®4 and PamChip®96 array plates used for kinase activity profiling. **B**. The reaction takes place on peptides spotted on the array. Peptide phosphorylation through active kinases, or inhibition thereof, can be monitored in lysates of cell lines or tumor tissues by fluorescent readout, using a set of anti-phospho-ser/thr specific antibodies and a fluorescently labeled secondary antibody for detection.

Prediction of response to MTKI

As shown previously [1], inhibition of tyrosine phosphorylation by a multi tyrosine kinase inhibitor (MTKI, main target EGFR) was able to predict response or resistance of cells both in a proliferation assay and in xenografts. Here, phosphorylation by ser/thr kinases was investigated for the same 24 cell lines. Basal phosphorylation profiles obtained on the PamChip® peptide microarray did not correlate with sensitivity in the proliferation assay. Ratios of phosphorylation in the presence and absence of inhibitor UCN-01 (hydroxystaurosporin, a PKC/PDPK inhibitor), were calculated. Inclusion of all cell lines in the analysis did not yield a classifier to predict response in the proliferation assay. As described previously [1], cell lines harbouring mutations in the Ras or PTEN pathways that confer resistance to MTKI, had to be excluded.
Inhibition ratio's for the remaining cell lines are depicted in Fig 3. Each column represents a cell line, each row a peptide. More inhibition (red) was observed in responder cell lines. Class prediction analysis of the cell lines was performed using partial least squares discriminant analysis (PLS-DA). Fig. 4 shows the result of evaluating the classifier using leave one out cross validation. The missclassification rate was 23 %.
Similar experiments were performed with spiking in the inhibitors flavopiridol (a CDK inhibitor) and AZD6244 (a MEK inhibitor). In neither case, a classifier for the prediction of response to MTKI could be identified.

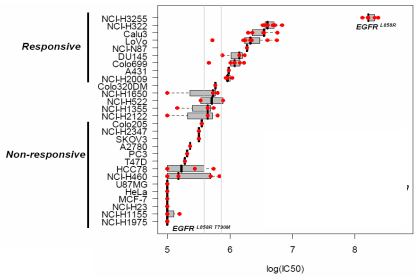

Fig. 2. Inhibition of cell proliferation by MTKI in 27 cell lines. Each dot represents an individually determined IC50. Cell lines are divided in responder and nonresponder cell lines [1].

The PTK peptide signature that predicts response to MTKI contains many EGFR and src substrate and signalling peptides. The EGFR pathway leads to activation of PI3K/Akt/mTOR pathway. Therefore, activity in this pathway is expected to be higher in the responder cell lines. Spiking in of an inhibitor of this pathway may therefore cause more inhibition in the response cell lysates. UCN -01 is an inhibitor of PDPK, the kinase that activates Akt [2].

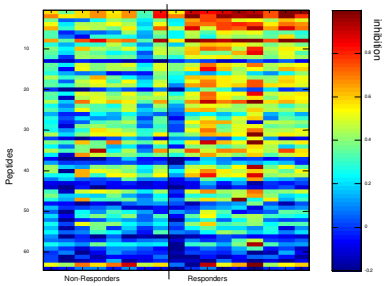

Fig 3. Classification of cell lines based on inhibition profiles obtained by spiking in the inhibitor UCN-01. Each horizontal line represents a peptide. The color map shows the UNC-01 inhibition profiles (log2 inhibition ratios were positive values indicate more inhibition). Each column represents a cell lysate. Signals are averaged per array and per peptide. Samples are sorted along the x-axis according to response, peptides are sorted along the y-axis according to the value of respective t-statistic for the difference between responder and non-responder samples

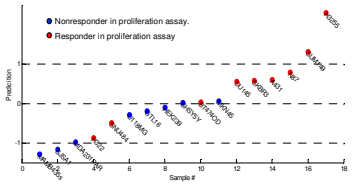

Fig 4. PLS class prediction results for 17 cell lines based on double Leave One Out Cross Validation.

H3255, a cell line that harbours the activating mutation L858R, is most sensitive to MTKI in het proliferation assay, and most sensitive to UCN-01. Two misclassified responding cell lines have high EGFR activity and would be expected to also have high inhibitable PDPK/Akt activity. The misclassified nonresponder has a high Met activity, and is there fore not expected to respond to MTKI.

References

1. Versele et al. Mol Cancer Therapeutics 2009; 8(7), 1846-1855.
2. Sato et al, Oncogene 2002, 21, 1727-1738

Conclusions

- Peptide microarrays can be used to predict response to treatment as exemplified for cell lines in a proliferation assay. Prediction of response can be based on inhibition profiles of both tyrosine kinases activity and ser/thr kinase activity. The multiparameter readout of kinase activity links response prediction to activity of relevant signaling pathways by the appropriate choice of kinase inhibitors.
- These results show that the effect of inhibitors on kinase activity can be determined in a complex cellular environment (data not shown).
- Our *in vitro* assay is a promising tool which, in future, can serve as a companion diagnostic test. Response to several kinase inhibitors can be tested on patient biopsy material which will aid in tailoring treatment to each individual patient.