Kinase activity based biomarkers: Identification of prognostic and erlotinib response prediction markers in NSCLC patients

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Abstract

Background

Reliable diagnostic tests are needed to identify early stage non-small cell lung cancer (NSCLC) patients with poor prognosis. Concomitantly there is a clear need for tests that enable the selection of patients who will benefit from targeted therapy with kinase inhibitors. We explored kinase activity profiles in two groups of early stage NSCLC patients, either for prognosis of long- or short-term survival, or for predicting of erlotinib drug response.

Method

Retrospective studies were performed on fresh frozen resection material of two groups of early stage NSCLC patients. The first group consisted of 48 short- and long-term survivors who underwent a complete surgical resection (6+ years follow-up). The second group consisted of 14 NSCLC patients who received 3 weeks of neo-adjacent treatment with erlotinib prior to complete surgical resection. Response evaluation to neo-adjacent treatment was based on histopathological examination of the surgical specimens. For both studies, kinase activity profiles of protein tyrosine kinase inhibitors on PamChip® peptide micro-arrays, comprising 144 tyrosine containing peptides derived from known phosphorylation sites of human proteins. Partial least square discriminant analysis was used to construct prediction models. CustAlV alignment algorithms were used to investigate the most informative phosphorylation sites.

Results

Kinase activity profiles obtained in the absence of inhibitor did not distinguish between subgroups (long- versus short-term survival, responder or non-responder to TKI), whereas ratios of inhibited versus non-inhibited signals resulted in distinct classifiers predicting survival for the first group, and response for the second group. Multivariate unsupervised analysis with leave-one-out cross-validation resulted in an error rate for survival prediction of 29%. In the drug response prediction 1 responder out of 14 patients was misclassified.

Conclusion

This is the first study to show that kinase activity profiles of tumor tissue exposed to a kinase inhibitor can be used to identify NSCLC patients likely to respond to treatment. Furthermore, based on kinase activity profiles of early stage NSCLC tumors, a prognostic classifier, for a set of 48 patients, was obtained.

Identification of prognostic biomarkers

A retrospective study was performed on two groups of early stage NSCLC patients (stage IA-IIIA) who underwent a complete surgical resection. The groups consisted of short term survivors and long term survivors (survival duration from diagnosis: 24 months (n=22) to 48 months (n=26). Follow-up was at least 5 years for all patients.

Phosphorylation profiles obtained in the absence of gefitinib did not distinguish between patients with short survival and long survival, whereas ratios of inhibited vs. non-inhibited signals resulted in a classifier able to predict survival. Interestingly, the inhibitory effects of gefitinib were stronger in patients with short survival times compared to patients with long survival times (fig.1).

Multivariate unsupervised analysis (fig.2) with leave-one-out cross-validation resulted in the correct prediction of outcome for 33 out of 48 patients (71%).

Identification of predictive biomarkers

Investigations were performed on 14 samples of early stage (stage IA-IIIA) NSCLC patients included in the M06NEL study (fig.3). This is an open label, multicenter, non-comparative phase II study.

Multivariate unsupervised analysis (fig.2) with leave-one-out cross-validation resulted in the correct prediction of outcome for 33 out of 48 patients (71%).

Phosphorylation profiles in the presence and absence of inhibitors. Per peptide, the ratio of inhibited/non-inhibited was calculated. A treatment prediction score is shown in figure 5.