**PamGene’s Kinase activity profiling of tumor tissues of different origin**

**Introduction:**
Many new anti-cancer drugs target kinase activity. Unfortunately, methods to monitor the drug effects are at the enzymatic level in patient derived tumor tissue are limited. Here, a novel molecular profiling method for application in biomarker discovery is presented that is based on measuring kinase activities in tumor tissue extracts. An *in vivo* activity-based approach which involves assessment of inhibition by a drug of interest, is illustrated for leukemia, locally advanced rectal cancer, lung cancer and melanoma tumors. The discovery of prognostic and predictive biomarkers is illustrated with case studies of LARC and breast and lung cancer. Here we present the applicability of this approach for biomarker discovery in multiple tumors of different origin.

**Technology:**
All these studies are enabled by dynamic peptide PamChip® microarrays comprising of peptides, which are known substrates for phosphorylation by protein kinases. A PamStation®X2 enables 12 microarray incubations per run and the PamChip® plate format enables 96 runs per plate. The PamChip® disposable consists of 4 identical arrays, each array containing up to 144 peptides immobilized on a porous ceramic membrane. The 13 amino acid peptide sequences harbor phosphorylation sites derived from literature or computational predictions and are correlated with one or more upstream kinases, allowing for multiplex measurements. Fluorescently labelled anti-phospho antibodies are used to detect phosphorylation activity of kinases present in the assay sample. During the assay, the sample solution is pumped up and down the porous membrane allowing for faster kinetics and real-time measurements. When the solution is underneath the array, the CCD camera in the workstation takes an image of each array which are later used by the BioNavitar® software to generate kinetic data curves of each peptide. The data workflow consisting of image quantification, quality control, statistical analysis, visualization and interpretation is performed using the BioNavitar® software.

**Methods:**
Patient-derived fresh frozen tissues of different tumor types (xenograft tissue for the breast cancer example) are used for extraction of total protein in Tris buffer. Equal amounts of total protein are analyzed for kinase activity on dynamic PamChip® peptide microarrays. Protein amounts ranging from 1 - 5 μg are used per microarray analysis. Tumor extracts are analysed in the presence and absence of kinase inhibitor drugs added ex vivo in the assay (E.g.: L-685,458, LARC, suinibib, NSCLC, gefitinib; Melanoma, vemurafenib) or in vivo in xenograft models (E.g.: Breast cancer bevacizumab and or doxil®).

**Results:**
We show here that from different tumors ATP-dependent kinase activity profiles could be generated. In addition, inhibitor dependent modulation of the peptide phosphorylations can be observed in all case studies described. In the leukemia study, concentration dependent inhibition is shown. Furthermore, differential kinase activity profiles were obtained that could be correlated to different patient subgroups. For example, in the LARC example, suinibib inhibition profiles could classify tumors into DTC (-) or (+) phenotypes. In Lung cancer, correlation to clinical data (long vs short term survival) prediction is clearly evident. In the melanoma study, vemurafenib inhibition profiles could distinguish BRAF v600E mutant samples from wild-type. The breast cancer study shows potential of combination therapy predictions in clinical samples.

**Conclusion:**
A novel molecular profiling approach was successfully applied for classification of different tumors. This approach is based on detection of kinase activities as well as inhibition of kinase activity in tumor tissues. Application of this method in the discovery of biomarkers for diagnosis, prognosis or prediction of drug response is foreseen.

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