Kinase activity profiling of tumor tissues of different origin

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Introduction:

Many new anti-cancer drugs target kinase activity. Unfortunately, methods to monitor the drug effects at the enzymatic level in patient derived tumor tissue are lacking. Here, a novel molecular profiling method for application in biomarker discovery is presented. This method is based on measuring kinase activities in tumor tissue extracts, and involves assessment of inhibition by a drug of interest. This ex vivo activity-based approach is enabled by dynamic peptide microarrays. These arrays comprise of peptides, which are known substrates for phosphorylation by protein kinases. Here we investigated the applicability of this approach in the classification of multiple tumors of different origin.

Method:

Patient-derived fresh frozen tissues of different tumor types (24 breast cancer (BC), 14 non-small cell lung cancer (NSCLC), 12 renal cell carcinomas (RCC)) were used for extraction of total protein in lysis buffer. Dependent on the tissue source, 6 slices of 10 µm were sufficient for 20-100 microarray analyses. Equal amounts of total protein were analyzed for kinase activity on dynamic PamChip peptide microarrays. These arrays comprised 144 or 256 different peptides. A PamChip96 plate format was used, enabling 96 microarray incubations per run. Protein amounts ranging from 1 - 5 µg were used per microarray analysis. Tumor extracts were analysed in the presence and absence of kinase inhibitor drugs (RCC: sorafenib and sunitinib, NSCLC: gefitinib).

Results:

We show here that from different tumors kinase activity profiles could be generated reproducibly (10-15% CV for selected peptides). Control experiments showed protein concentration and ATP dependency. In the RCC experiments different phosphorylation profiles were obtained when the non-tumor tissue was compared to tumor tissue derived from the same patient, showing higher kinase activities in the latter. In addition, inhibitor dependent modulation of the peptide phosphorylations was observed (sorafenib vs. sunitinib). Furthermore, differential kinase activity profiles were obtained that could be correlated to different patient subgroups (BC) or clinical data (long vs. short term survivors; NSCLC).

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.

Information:

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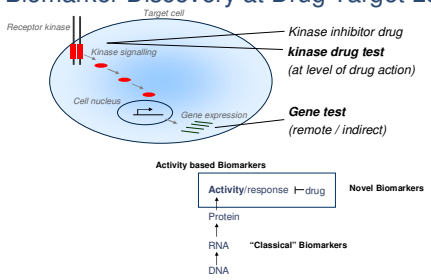
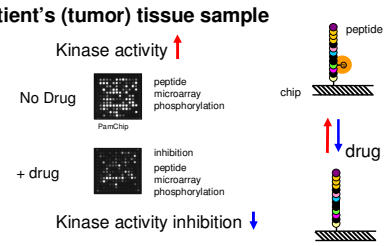
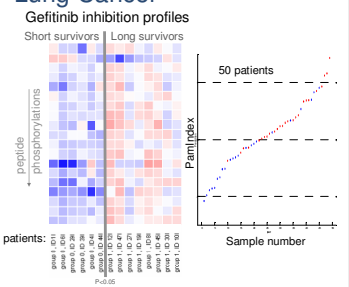
Biomarker Discovery at Drug Target Level



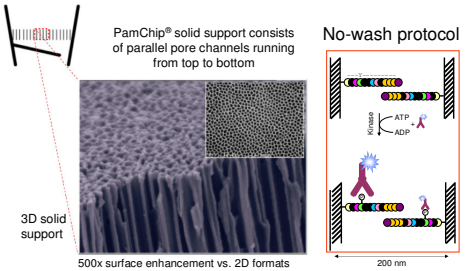
Drug testing on tissue derived kinases

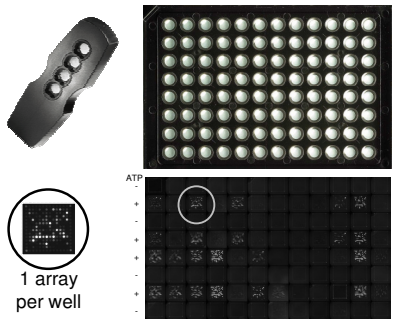


Lung Cancer

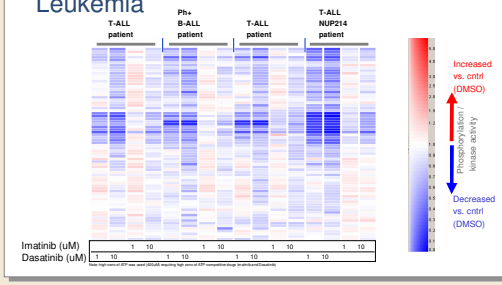


Dynamic Peptide Microarray Technology

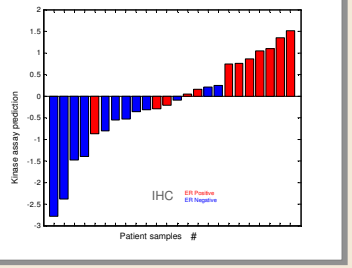




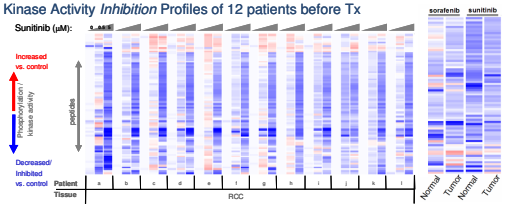
Leukemia



Breast Cancer



Renal Cancer



Reproducibility

Technical coefficient of variation (CV) of high intensity signals	# patients (N)	CV(%)
Cell line		
Various	NA	12.8
Tumor tissue		
NSCLC (resection material)	14	9.7
Ovarian cancer (resection material)	24	15.0
CML (bone marrow samples)	15	8.9
Rectal Cancer (biopsies)	72	8.7

Kinetic Readout