Tyrosine-kinase activity profiling of tumor tissues from Renal Cell Carcinoma patients

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Introduction: Treatment of patients with metastasized renal cell carcinoma (RCC) with Tyrosine Kinase Inhibitors (TKI) leads to disease stabilization and disease response in a substantial percentage of patients. The increase in progression-free survival is considerable, but the effect on over-all survival is less clear. Additionally, many patients experience mild to severe toxicity, which may even lead to dose reduction. In view of the heterogeneous response it would be beneficial to stratify patients to delineate e.g. responder *versus* non-responder patients. To achieve this goal of subclassification of patients based on testing tissue material obtained *before* they will be treated with the TKI, response biomarkers need to be identified. The biomarker method used here is based on kinase activity profiling which is at the biological level these drugs act (fig 1, 2 and 3).

Materials and Methods: Tissue was collected immediately after surgery, snap-frozen and stored at -80° C until use. Approximately 50-100 mg tissue was lysed and tyrosine kinase activity of paired normal kidney tissue and RCC tissue was measured on PamChip® Peptide micro-arrays (fig 4) using 5-7 ug of total protein per test, containing 144 peptides derived from known phosphorylation sites in Protein Tyrosine Kinase substrates.

Results: RCC showed unique, individually distinct tyrosine kinase activity patterns (fig 5a), with 4/5 RCC demonstrating much higher kinase activity then the parallel normal kidney tissue. Phosphorylation of multiple peptides, e.g. derived from platelet-derived growth factor B (PDGFB) differed significantly between RCC and normal kidney. Initial analysis suggested segregation of RCC using conditional clustering.

Conclusions and Discussion: Our results show that tyrosine kinase activities of individual RCC specimens can be measured accurately with this chip technology. Importantly, amongst peptides that differed between RCC and normal kidney, tyrosine kinase protein targets of which phosphorylation is known to be upregulated in RCC (e.g. PDGFB) were identified, indicating that this approach could identify appropriate target proteins. Despite the difference in individual tyrosine kinase activity, it appears that stratification of RCC with different kinase activity can be achieved. This suggests that it may be possible to stratify RCC patients based on their tyrosine kinase activity profile. Such stratification might be helpful in guiding treatment strategies. We are currently studying the effects of TKI on the tyrosine kinase activity *in vitro* (fig 5b and c), which might allow us to guide the selection of TKI treatment.

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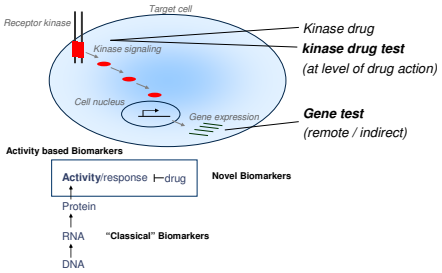
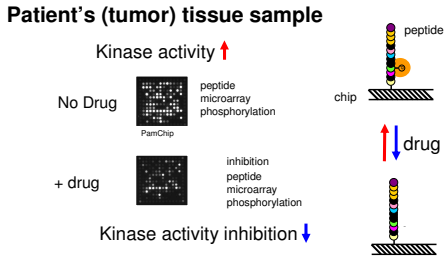
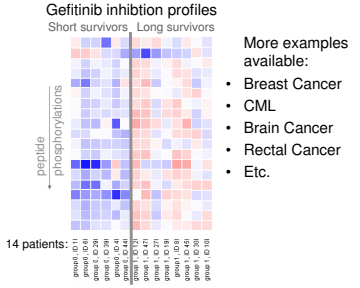
1. Biomarker Discovery at Drug Target Level



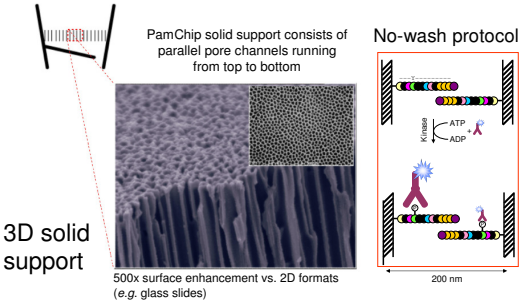
2. Drug testing on Patient's derived kinases

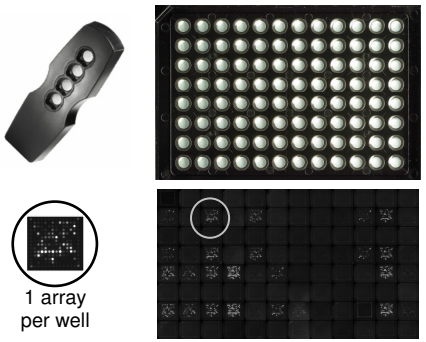


3. Example: Lung Cancer



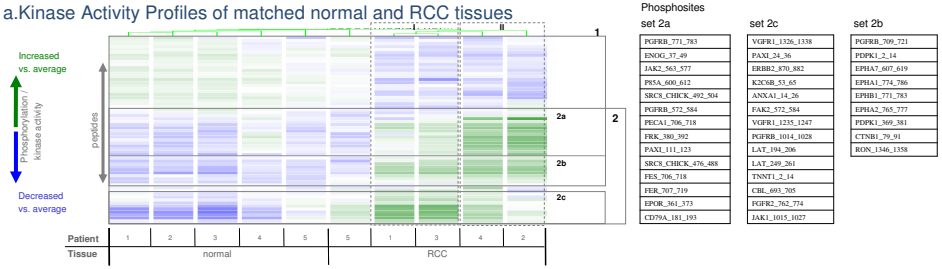
4.Dynamic Peptide Microarray Technology



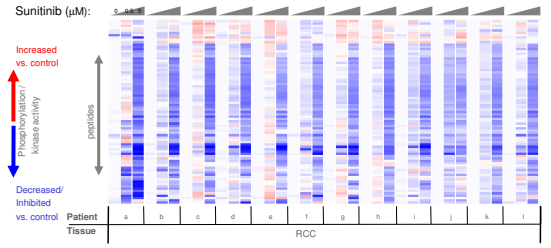


5.Results

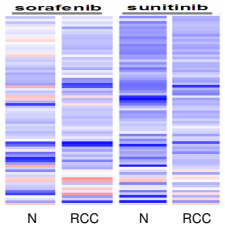
a.Kinase Activity Profiles of matched normal and RCC tissues



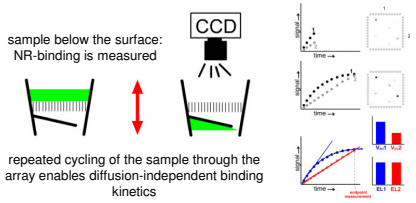
b. Kinase Activity *Inhibition* Profiles of 12 Sunitinib treated patients



c. Patient (81): TKI inhibition profiles



Kinetic Readout



- Increased kinase *activity* detectable in tumor tissue vs. matched normal kidney tissue 4/5 patients (5a)
- Segregation of RCC based on kinase activity profiles (5a)
- Dose dependent Sunitinib inhibition profiles in 12 out of 12 patients tested (5b)
- Example of similar inhibition profiles of kinase activities by Sorafinib and Sunitinib in tumor tissue, but distinct differences (off-target effects?) in normal tissue from the same patient. (5c)