Direct detection of AKT/PKB activity in a Pten knock out mouse model using dynamic peptide microarrays.

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Background

In many cancers, deregulation of kinases or phosphatases leads to activation of the AKT/PKB pathway. This pathway regulates many cellular processes including metabolism, differentiation, translation, proliferation and apoptosis, so is central in determining cell fate. In addition, this pathway is often hyperactive in tumor cells resistant to kinase inhibitor therapies (survival pathways). Several kinase inhibitors that influence AKT activity are investigated in the context of new pharmacotherapies in cancer. However, often a fraction of patients responds and it is not possible to identify those patients up front. It would be a major leap forward if response to therapy could be determined in an in vitro assay prior to the onset of therapy. Methods to directly measure this activity do not exist.

In this study, we explored:
1) Whether it is feasible to determine AKT activity profiles in lysates of hyperplastic prostates of PTEN knock-out mice (PSA-Cre;Pten-loxP/loxP mice).
2) Whether addition of AKT inhibitors affects the phosphorylation profiles in an inhibitor specific way.

Presence of Akt in Pten knockout mice

Activity of Akt in Pten knockout mice

PamChip® Cell-Based Kinase Assay

A
B
C

Fig. 1. A. PamChip® 96 microarray plate and peptide microarray used for kinase activity profiling. The solid support is three-dimensional and consists of pores in which the peptides are immobilized. B. 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. C. The signals per peptide are quantified and analysed using Biomat software.

Methods

The PSA-Cre;Pten-loxP/loxP mouse prostate cancer model was described in [1] and [2]. Prostate tissues of three 4 months old PSA-Cre;Pten-loxP/loxP mice and three wt mice were lysed in MPER buffer with protease and phosphatase inhibitors. IHC and western blotting were performed using standard methods. Lysates and recombinant full length AKT1 were incubated at least in triplicate on PamChip® peptide micro-arrays [3] comprising 256 ser/thr containing peptides derived from known human phosphorylation sites. Per array, 0.5 μg of protein was applied. As controls, incubations without ATP were performed. Peptide phosphorylation was monitored with a mixture of 6 anti-phosphoserine and anti-phosphothreonine antibodies. The effect of AKT inhibitors MK2206 and AKT Inhibitor VIII and non-AKT inhibitor UCN-01 (hydroxytaurospermine) on phosphorylation profiles was tested at 18 h, 50 uM and 10 uM respectively.

Conclusions

We measured AKT activity in a Pten knock-out (PSA-Cre;Pten-loxP/loxP) mouse prostate tissue lysate, using a multiplex kinase assay. The activity of kinases in this tissue was increased compared to wild type tissue levels. In the presence of AKT and non-AKT inhibitors, differential modulation of kinase activity was observed. The activity profiles of the PTEN/AKT pathway will support the studies on the complicated regulation of AKT activity. This new multiplex kinase activity assay will add a new dimension and a scope of applications like drug testing directly in patient derived tissue (biopsies). Detection of a-priori active resistance pathways in pre-dose biopsies is such an application with relevance to drug response prediction.

References