ALK F1174L kinase activity as driver of cell proliferation in neuroblastoma cell line models and neuroblastoma tumors

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Background

Whereas ALK translocations play a role in tumor growth in selected NSCLC cases, point mutations in ALK have been shown to drive development of neuroblastoma, the most common malignant extracranial tumor of childhood (1-4). The mutation F1174L in the oncogene ALK increases the kinase activity, causing deregulated proliferation of sympatho-adrenal progenitor cells. We explored the use of kinase activity measurements to identify ALK activity in cell lysates and patient derived tissue lysates with the aim of identifying biomarkers to trace the activity of ALK in tumors and the effect of ALK inhibitors on these marker signals.

PamChip® Cell-Based Kinase Assay

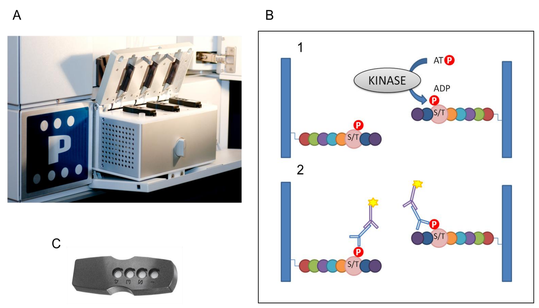

Fig. 1. **A**. PamStation®4, 12 and 96 instruments and PamChip®4 and PamChip®96 array plates used for kinase activity profiling. **B**. The reaction takes place on peptides spotted on the array. Peptide phosphorylation through active kinases, or inhibition thereof, can be monitored in lysates of cell lines or tumor tissues by fluorescent readout, using either an anti-phosphotyrosine antibody or a set of anti-phospho-ser/thr specific antibodies and a fluorescently labeled secondary antibody for detection.

Validation of ALK activity measurement

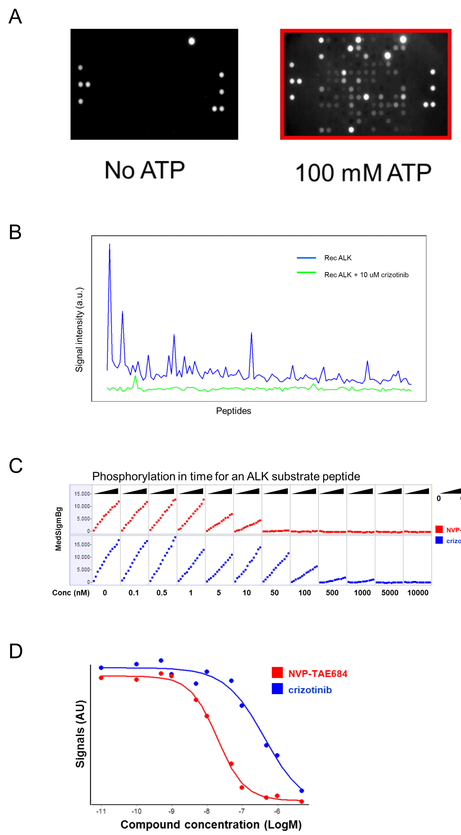

Fig 2 Validation of kinase activity measurement. A.Signal intensity depends on the presence of ATP and increases with increasing ALK concentration (data not shown). B. Phosphorylation profile without and with ALK inhibitor crizotinib. C. Signals on ALK substrate peptides increase in time and are inhibited by ALK inhibitors. D. IC50 curve for Rec ALK based on signal intensities after 60 min of incubation.

ALK activity in cell lines and tumors

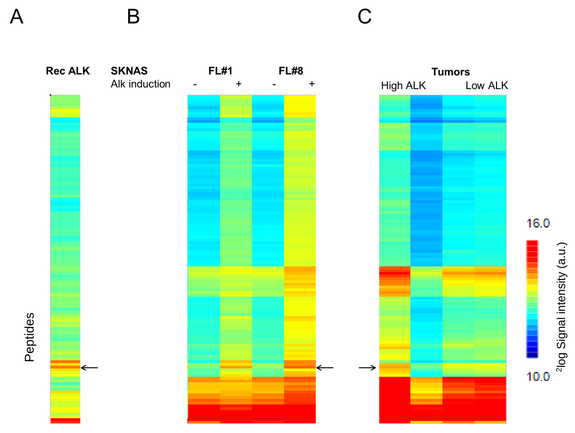

Fig. 3. ALK kinase activity for recombinant kinase, (A) in cell lines (B) and in high and low ALK expressing tumors (C).

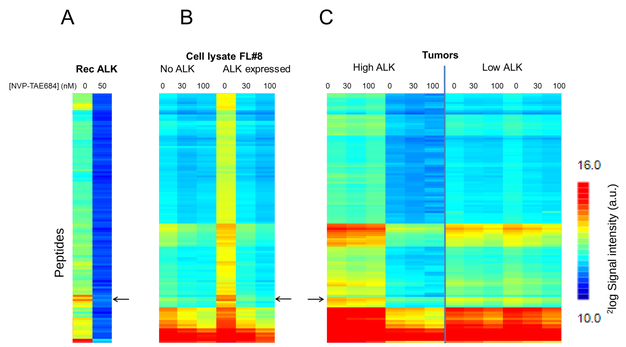

Fig. 4. Effect of ALK inhibitor NVP-TAE684 on signal intensities of rec ALK (A), cell lysates overexpressing ALK (B) and tumor lysates with high and low ALK. (C).

Methods

Protein kinase activities were measured on PamChip® peptide microarrays comprising 144 Tyr containing peptide sequences from known human phosphorylation sites. The activity profiling experiments involved recombinant ALK kinase, lysates of SKNAS cell lines overexpressing ALK and neuroblastoma tumors with elevated ALK F1174L presence. Per microarray analysis 50 ng of rec ALK or 5 ug of total protein was used in the presence of 100 uM ATP. Experiments were performed in the presence and absence of ALK kinase inhibitors crizotinib and NVP-TAE684. Real time kinetics of 144 peptide phosphorylations were measured with a fluorescently labeled anti-phosphotyrosine antibody. Signal intensities were corrected for signals at the start of the incubation.

Conclusions

ALK activity of recombinant kinases, cell lysates and tumor lysates can be detected on PamChip® peptide microarrays. Peptides indicative of increase ALK activity have been identified both in cell lysates and a tumor with high ALK expression. Based on results with model systems, this method shows promise to identify tumors that express elevated ALK activity and to study response of this class to ALK inhibitors.

For recALK 48 novel peptide substrates were identified that showed signals depending on both ATP and kinase concentration and inhibition by ALK inhibitors. In ALK overexpressing cell lines, but not in control cell lysates, signals on many ALK substrate peptides were significantly elevated, and returned to baseline level in the presence of ALK inhibitors (Fig 3.B and 4B). Whereas neuroblastomas with low ALK expression gave similar kinase activity profiles, of the tumors harboring the ALK F1174L mutation one had high ALK activity, the other low activity, suggesting a more complex regulation of phosphorylation activity in these tumors (Fig 3C and 4C). One ALK substrate peptide shows elevated signal in one of the high ALK tumors. All tumors showed on-chip responses with ALK inhibitor drugs, but to a different extent.

References

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