Kinome Profiling in Pediatric Brain Tumors; a New Approach for Target Discovery

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Study Design

PamChip® tyrosine kinase activity profiles of 29 pediatric brain tumors (9 ependymomas, 7 pilocytic astrocytomas, and 13 primitive neuroectodermal tumors, of which 10 medulloblastomas) were generated. From each tumor, 12 slices of 5 µm of a 5x5mm tissue block was lysed.

Key Findings

Figure 1 shows a supervised clustering of the 4 different brain tumor types. The vendiagram in figure 2 shows that 30 substrates are common for all pediatric brain tumor types. 20/30 identified peptides are potentially phosphorylated by Src family kinases. The hypothesis of Src being the common kinase, was confirmed by Phos-Tag SDS-PAGE approach (figure 3) . This technique makes use of the affinity of phosphorylated proteins for the Phos-Tag ligand, resulting in retention of phosphorylated proteins, separating them from their unphosphorylated counterpart during electrophoresis. Src kinase activation in tissue samples of all three brain tumor as well as in each of 9 pediatric brain tumor cell lines was confirmed through Phos-Tag SDS-PAGE (Sikkema et al. 20009).

"Author Quote"

In this study, we successfully applied a novel high-throughput technique to generate tyrosine kinase activity profiles of pediatric brain tumors and show its usefulness in target discovery. We identified and validated Src activity as a potential target in pediatric brain tumors for therapeutic intervention.

Background

Progression in pediatric brain tumor growth is thought to be the net result of signaling through various protein kinase mediated networks driving cell proliferation. Defining new targets for treatment of human malignancies, without a priori knowledge on aberrant cell signaling activity, remains exceedingly complicated. Here, we show kinome profiling using flow-through peptide microarrays as a new concept for target discovery.

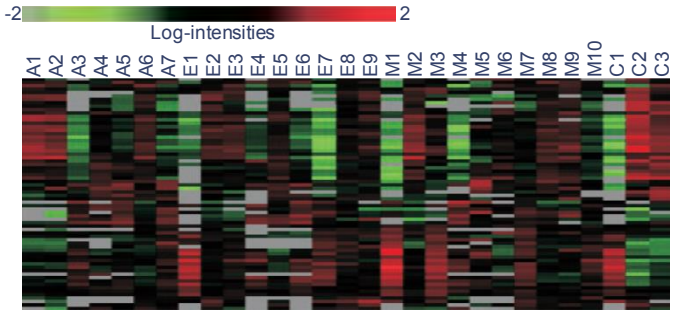

Figure 1: Supervised clustering of normalized peptide phosphorylation signals. The 68 peptides that were phosphorylated by >60% of all brain tumor lysates are displayed. Red and green spots, high and low signal intensities, respectively. (A, astrocytoma; E, ependymoma; M, medulloblastoma; C, central nervous system primitive neuroectodermal tumor).

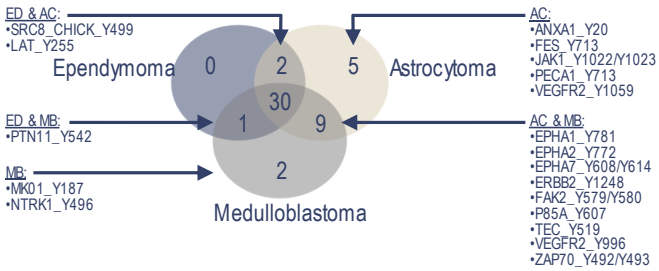

Figure 2: Peptides phosphorylated by astrocytoma, medulloblastoma, and ependymoma tissue lysate. The number and identity of the peptides phosphorylated within one tumor type are displayed in a Venn diagram. A remarkable 30 peptides were phosphorylated by each type of pediatric brain tumor.

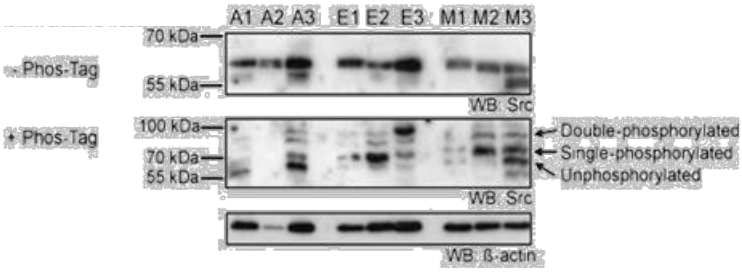

Figure 3: Src phosphorylation levels were determined and confirmed in three tissue samples of each brain tumor type using Phos-Tag SDS-PAGE. Three phosphorylation states representing unphosphorylated as well as single- and double-phosphorylated Src can be identified. Src kinase phosphorylated at both Tyr416 and Tyr527 renders a hyperactive isoform. Hence, the blotting results confirm substantial Src kinase activation. (A, astrocytoma; E, ependymoma; M, medulloblastoma; C, central nervous system primitive neuroectodermal tumor).

References:

Sikkema AH. et al., Cancer Res 2009; 69: (14). July 15, 2009.

Conclusion

PamChip® PTK peptide arrays, were successfully employed as a kinase activity screening technique which allows identifying of new targets for pediatric brain tumor treatment.

Application note: #201008 (10-2010)

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