

Oncogenic ALK signaling in neuroblastoma

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ABSTRACT

Over the last decade Anaplastic Lymphoma Kinase (ALK), a receptor tyrosine kinase (RTK) has been identified as a translocation partner in diverse cancer types. In tumors, where the full-length ALK RTK itself is mutated, such as neuroblastoma, the picture is less clear regarding ALKs role as an oncogenic driver. Neuroblastoma is a heterogeneous disease of the sympathetic nervous system, accounting for 10-15% of all childhood cancer deaths. A number of small tyrosine kinase inhibitors (TKIs) have been developed to inhibit ALK activity. The data acquired thus far suggests that ALK TKI mono-treatment may not be as effective solution for ALK positive neuroblastoma patients. Therefore, there is a need for combination therapy using drugs towards different targets or signaling pathways to combat the disease. The overall aim of this thesis is to identify targets in signaling pathways that can be inhibited by specific drugs, as a potential poly-therapy treatment strategy in ALK positive neuroblastoma patients.

Using an MS-based phosphor-proteomics approach, we identified STAT3 as a potential downstream target of oncogenic ALK signaling (Paper I). ALK activation of STAT3 results in increased phosphorylation of STAT3 in PC12 cells expressing a gain-of-function ALK mutation. Pharmacologic inhibition of STAT3 using FLLL32 and STATTIC resulted in decreased phosphorylation levels of STAT3 and *MYCN* protein and mRNA levels. This study identified STAT3 as a target of ALK signaling and showed that inhibition of STAT3 using FLLL32 and STATTIC decreases proliferation of neuroblastoma cell lines and regulates the transcription of *MYCN*.

In a subsequent paper, we identified ERK5 as a potential ‘druggable’ target for ALK positive neuroblastoma patients (Paper II). Inhibition of ERK5 activity, reduced proliferation of ALK positive neuroblastoma cells as well as *MYCN* mRNA levels. Combination of ALK and ERK5 inhibitors abrogated tumor growth and cell proliferation synergistically. Overall, this study showed that ALK activates ERK5 via the PI3K pathway and regulates *MYCN* transcriptionally, suggesting that targeting both ALK and ERK5 might be beneficial for ALK positive neuroblastoma patients.

In paper III, we addressed whether MEK inhibition alone or in combination with ALK inhibitor(s) has therapeutic value in a large panel of neuroblastoma cell lines. MEK inhibition alone in ALK positive neuroblastoma cells or xenografts did not abrogate cell or tumor growth. We showed that pharmacological inhibition of MEK-ERK pathway in ALK-positive neuroblastoma cells results in increased levels of activation/phosphorylation of AKT and ERK5. This feedback response is regulated by the mTOR complex 2 protein SIN1. Our results contraindicate the use of MEK inhibitors as effective therapeutic strategy in ALK-positive neuroblastoma.

Together, this study highlights the importance of full length ALK receptor signaling in neuroblastoma. Further, it shows that combination of ALK inhibitor with PI3K/Akt/mTOR/ERK5 pathway inhibitors might be a potential therapeutic treatment strategy for ALK positive neuroblastoma patients.

Keywords: Neuroblastoma, Anaplastic Lymphoma Kinase, Akt, ERK5, mTOR, MYCN

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Av

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Avhandlingen baseras på följande arbeten:

- I. Phosphoproteomic analysis of anaplastic lymphoma kinase (ALK) downstream signaling pathways identifies signal transducer and activator of transcription 3 as a functional target of activated ALK in neuroblastoma cells.
Sattu K, Hochgräfe F, Wu J, **Umapathy G**, Schönherr C, Ruuth K, Chand D, Witek B, Fuchs J, Li PK, Hugosson F, Daly RJ, Palmer RH, Hallberg B. FEBS J. 2013 Nov;280(21):5269-82. doi: 10.1111/febs.12453.
- II. The kinase ALK stimulates the kinase ERK5 to promote the expression of the oncogene MYCN in neuroblastoma.
Umapathy G, El Wakil A, Witek B, Chesler L, Danielson L, Deng X, Gray NS, Johansson M, Kvarnbrink S, Ruuth K, Schönherr C, Palmer RH, Hallberg B. Sci Signal. 2014 Oct 28;7(349):ra102. doi: 10.1126/scisignal.2005470.
- III. Anaplastic lymphoma kinase addictive neuroblastoma cell lines are associated with growth upon treatment with MEK inhibitor trametinib.
Umapathy G, Gustafsson DE, Javanmardi N, Madrid DC, Martinsson T, Palmer RH, Hallberg B. (Manuscript)



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