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BioIDentification of Alk-associated signaling complexes

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet, kommer att offentligen försvaras i Europa, Konferenscentrum, Wallenberg, Medicinaragatan 20A, Göteborg, den 25 november 2022, klockan 13:00.

av Ezgi Uçkun

Fakultetsopponent:
Prof. Marta Miączyńska
International Institute of Molecular and Cell Biology in Warsaw, Poland

Avhandlingen baseras på följande delarbeten

I. **Uçkun E**, Siaw JT, Guan J, Anthonydhason V, Fuchs J, Wolfstetter G, Hallberg B, Palmer RH. BioID-screening identifies PEAK1 and SHP2 as components of the ALK proximitome in neuroblastoma cells. *J. Mol. Biol.* (2021)

II. **Uçkun E**, Wolfstetter G, Anthonydhason V, Sukumar SK, Umapathy G, Molander L, Fuchs J, Palmer RH. *In vivo* profiling of the Alk proximitome in the developing *Drosophila* brain. *J. Mol. Biol.* (2021)

III. **Uçkun E**, Pfeifer K, Guan J, Wolfstetter G, Anthonydhason V, Palmer RH. Proximity labeling identifies regulators of Alk signaling in the *Drosophila* CNS. *(Manuscript)*

SAHLGRENSKA AKADEMIN

INSTITUTIONEN FÖR BIOMEDICIN

GÖTEBORGS UNIVERSITET

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BioIDentification of Alk-associated signaling complexes

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Gothenburg, Sweden, 2022.

Abstract

Anaplastic lymphoma kinase (Alk) is a receptor tyrosine kinase (RTK) of the insulin receptor family. Alterations in human ALK signaling have been implicated in multiple malignancies including pediatric neuroblastoma. In addition to its role in oncogenesis, previous studies on both invertebrate and vertebrate model organisms have revealed a role for Alk signaling in the central nervous system including axon targeting, synapse development, growth and body size regulation, brain sparing, memory formation and learning, circadian rhythm, and longevity. Although the Alk receptor is associated with a wide range of processes, downstream components of Alk signaling are highly diverse and it is unclear how Alk signaling intersects with different downstream effectors, especially in different tissues. Analysis of Alk-associated signaling complexes in the context of wild-type, active and inactive Alk status in different tissues provides essential information, not only for the development of therapeutic approaches for targeting ALK-driven cancers, but also for understanding its role in neurodevelopmental processes. In this thesis, I have employed BioID-based proximity labeling (PL) to identify components of Alk signaling in both neuroblastoma (NB) cells (Study I) and the *Drosophila* larval brain (Studies II and III). In Study I, PL was performed in the presence or absence of ALKAL ligand stimulation as well as upon ALK inhibitor treatment. We identified PTPN11/SHP2 and PEAK1 as activity-dependent ALK interactors and functionally investigated the role of the protein tyrosine phosphatase SHP2 in ALK-addicted NB cells. In Study II, we defined the wild-type Alk proximitome by using three different BioID enzyme variants and identified the SHP2 ortholog Corkscrew (Csw) as a downstream component of Alk signaling. In the last study, we performed PL both in the presence or absence of Jeb ligand overexpression as well as in a gain-of-function *Alk* mutant and identified the LDL receptor related protein 4 (Lrp4) as a negative regulator of Alk activity in the *Drosophila* brain.

Keywords: ALK, neuroblastoma, proximity labeling, BioID, miniTurbo, TurboID

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