Ligand binding induces Cbl dependent EphB1 degradation through the lysosomal pathway

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Development of the Vascular System



Angiogenesis



- 1. Proliferation:
- VEGF receptor / VEGF
- FGF receptor / FGF
- PDGF receptor / PDGF

- 2. Targeting:
- Eph receptors/ephrins
- Tie receptors/angiopoetin

Erythropoeitin Producing hepatocellular Carcinoma



EphA1-A10

=> binding to ephrinA ligands

EphB1-B6 => binding to ephrinB ligands

Structure of Ephrins



Eph/ephrin Interactions

No proliferative functions
However, essential role in cell-cell and cell-matrix interaction

 \checkmark In the embryo, critical role in

- axonal guidance
- vascular patterning

Eph/ephrin Interactions

In the adult Eph receptors are upregulated in different kind of cancers.

However, regulation of their expression and function have still to be defined

How is Eph signaling terminated?

Signal Regulation of Receptor Tyrosine Kinases (RTKs)

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For a number of RTKs, signal termination is regulated by downregulation after ligand binding

Degradation of RTKs is known to occur in lysosomes but proteasomes can be additionally involved

EphB1 receptor is promptly degraded after stimulation with soluble ephrinB1/Fc



Biotinylation of cell surface proteins





Juxtacrine stimulation of CHO-EphB1 with CHOephrinB1 induces EphB1 degradation



EphB1 is degraded through the lysosomal pathway



IB: HA IB: actin EphrinB1/Fc - - 1h 1h 2h 2h 3h 3h Lactacystin - + - + - +

Lactacystin = proteasomal inhibitor



EEA-1 = early endosomal antigen 1

Ubiquitination



The c-Cbl adaptor protein: prototype of a RING ubiquitin ligase



TKB = tyrosine kinase binding domain RING = ubiquitin ligase activity

Ligand dependent EphB1 ubiquitination and phosphorylation of Cbl





Cbl co-localizes with activated EphB1



Negative Regulatory effect of Cbl on EphB1 protein levels







70Z-Cbl = mutation in the ubiquitin ligase domain

70Z

EphB1 recruits c-Src to activate MAPK/ERK



Vindis et al, *J Cell Biol* 2003

Src kinase mediates Cbl phosphorylation and EphB1 receptor degradation



Cbl phosphorylation

PP2 = Src family inhibitor PP3 = inactive homolg of PP2



An active tyrosine kinase domain is required for EphB1 degradation



Cbl is binding EphB1 through its TKB domain







Cbl is required for EphB1 ubiquitination





Model of EphB1 degradation



Fasen et al, *Traffic*, 2007