Cellular Signalling 27 (2015) 2209–2219

Contents lists available at ScienceDirect

Cellular Signalling

journal homepage: www.elsevier.com/locate/cellsig

Histidine-domain-containing protein tyrosine phosphatase regulates platelet-derived growth factor receptor intracellular sorting and degradation

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ARTICLE INFO

Article history:
Received 21 July 2015
Accepted 24 July 2015
Available online 30 July 2015

Keywords:
HD-PTP
PDGFRβ
STAT3
c-Cbl
Cbl-b
PLCγ
ESCRT
Ubiquitination

ABSTRACT

Histidine domain-containing protein tyrosine phosphatase (HD-PTP) is a putative phosphatase that has been shown to affect the signaling and downregulation of certain receptor tyrosine kinases. To investigate if HD-PTP affects platelet-derived growth factor receptor β (PDGFRβ) signaling, we employed the overexpression of HA-tagged HD-PTP, as well as siRNA-mediated and lentivirus shRNA-mediated silencing of HD-PTP in NIH3T3 cells. We found that HD-PTP was recruited to the PDGFRβ in a ligand-dependent manner. Depletion of HD-PTP resulted in an inability of PDGF-BB to promote tyrosine phosphorylation of the ubiquitin ligases c-Cbl and Cbl-b, with a concomitant missorting and reduction of the degradation of activated PDGFRβ. In contrast, ligand-induced internalization of PDGFRβ was unaffected by HD-PTP silencing. Furthermore, the levels of STAM and Hrs of the ESCRT0 machinery were decreased, and immunofluorescence staining showed that in HD-PTP-depleted cells, PDGFRβ accumulated in large aberrant intracellular structures. After the reduction of HD-PTP expression, an NIH3T3-derived cell line that has autocrine PDGF-BB signaling (sis-3 T3) showed increased ability of anchorage-independent growth. However, exogenously added PDGF-BB promoted efficient additional colony formation in control cells, but was not able to do so in HD-PTP-depleted cells. Furthermore, cells depleted of HD-PTP migrated faster than control cells. In summary, HD-PTP affects the intracellular sorting of activated PDGFRβ and the migration, proliferation and tumorigenicity of cells stimulated by PDGF.

1. Introduction

Platelet-derived growth factor (PDGF) is a family of potent mitogens for connective tissue cells that play important roles during embryonic development and wound healing. The PDGF family consists of four different polypeptide chains (PDGF-A, -B, -C and -D) making up five different biologically active dimers (PDGF-AA, -BB, -AB, -CC and -DD) [1]. The PDGF dimers function by binding to two structurally related receptor tyrosine kinases, i.e. PDGF receptor α and β (PDGFRα and PDGFRβ, respectively). The different receptors bind the ligands with different affinities; thus, PDGFRα binds PDGF-A, -B and -C, whereas PDGFRβ binds PDGF-B and -D chains [2]. Consequently, different homo- and heterodimeric receptor complexes may form depending on which PDGF isoform the cell is exposed to and which receptor isoform the cell expresses. The αα and ββ receptor homodimers, as well as the heterodimer, have overlapping, but to certain extent unique, signaling properties. In the dimeric state the PDGF receptors undergo autophosphorylation, which results in increased kinase activity, and provides docking sites for signal proteins with SH2-domains. Several signal transduction pathways are activated by the PDGF receptors, including phosphatidylinositol-3′-(PI3)-kinase, Src family kinases, phospholipase C (PLC) γ, STAT pathways and MAP kinase pathways [2]. Activation of these pathways ultimately results in increased cell proliferation, survival, migration or differentiation, depending on cell type and other external factors. Furthermore, overactive PDGF signaling has been observed in diseases, such as fibrosis, arthrosclerosis and certain types of malignancies.

Several processes limit the signaling output from activated PDGF receptors. It has been proposed that receptor ubiquitination is essential for its internalization and intracellular sorting toward degradation. The ubiquitin ligase c-Cbl has been implicated as particularly important for PDGFR ubiquitination [3,4]; the activity of c-Cbl may be regulated through tyrosine phosphorylation [5]. Another isoform of the Cbl family, Cbl-b, has

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http://dx.doi.org/10.1016/j.cellsig.2015.07.020
0898-6568/

also been found to participate in ligand-dependent PDGFR downregulation by interacting with the Cbl-interaction protein 85 (CIN85) [6]. Beyond receptor degradation, receptor dephosphorylation with subsequent reduction in its kinase activity can attenuate signaling. The histidine-domain-containing protein tyrosine phosphatase (HD-PTP), also denoted PTPN23, plays an important role in the endosomal sorting