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Posttranslational proteolytic processing of Leda-1/Pianp involves cleavage by MMPs, ADAM10/17 and gamma-secretase

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ABSTRACT

Leda-1/Pianp is a type I transmembrane protein expressed by CNS cells, murine melanoma cell line B16F10 and rat liver sinusoidal endothelial cells. The early steps of posttranslational modifications of Leda-1/Pianp have been described to include glycosylation and processing by proprotein convertases. Here, we comprehensively characterized the subsequent steps of proteolytic processing of Leda-1/Pianp. For this purpose specific protease inhibitors and cell lines deficient in PS1, PS2, PS1/PS2 and ADAM10/17 were deployed. Leda-1/Pianp was cleaved at numerous cleavage sites within the N-terminal extracellular domain. The sheddases involved included MMPs and ADAM10/17. Ectodomain shedding yielded C-terminal fragments (CTF) of ~15 kDa. The CTF was further processed by the γ (gamma)-secretase complex to generate the intracellular domain (ICD) of ~10 kDa. Although PS1 was the dominant intramembrane protease, PS2 was also able to cleave Leda-1/Pianp in the absence of PS1. Thus, Leda-1/Pianp is constitutively processed by proprotein convertases, sheddases including MMPs and ADAM10/17 and intramembrane protease γ-secretase.

1. Introduction

Leda-1/Pianp is a type I transmembrane protein expressed by CNS cells, murine melanoma cell line B16F10 and rat liver sinusoidal endothelial cells [1,2]. Notably, Leda-1/Pianp is also expressed in bone marrow derived macrophages of BALB/c but not of C57BL/6J mice [3]. The glycoprotein Leda-1/Pianp is constitutively cleaved by Furin-like proprotein convertases before its localization to the surface plasma membrane [2]. PILRα is so far the only known receptor that has been shown to be able to bind to Leda-1/Pianp. PILRα is primarily expressed in macrophages, dendritic cells and granulocytes. The binding of PILRα and Leda-1/Pianp depends on N-terminal sialic acid residues of Leda-1/Pianp [4]. Furthermore, PILRα and Leda-1/Pianp are contrarily regulated in murine myeloid cells upon LPS stimulation. Degradation of Leda-1/Pianp during the LPS response of RAW264.7 cells is mediated by proteolytic degradation by MMPs [3]. However, the physiological role of LPS mediated counter-regulation and the functional impact of their interactions during immune responses in vivo are currently not known.

As its only known homolog Ajap-1/Shrew1, Leda-1/Pianp is sorted to E-Cadherin positive adherens junctions at the basolateral side of the plasma membrane in polarized epithelial cells [1,5]. Basolateral sorting of Ajap-1/Shrew1 is mediated by C-terminal tyrosine residues [6]. In contrast, basolateral sorting of Leda-1/Pianp depends on a distinct juxtamembrane region containing several hydrophobic amino acid residues and leucine residues. Posttranslational modifications including glycosylation and proteolytic cleavage of Leda-1/Pianp also rely on this juxtamembrane region. Functionally, Leda-1/Pianp increases the transepithelial electrical resistance (TEER) of polarized MDCK cells indicating a function in barrier formation and stabilization. Furthermore, Leda-1/Pianp attenuated γ-secretase-mediated processing of E-Cadherin, which may contribute to junctional stabilization [5].

Overall these findings indicate that Leda-1/Pianp can be cell– and context-dependently either constitutively or inducibly cleaved by different proteases. However, the sequence of cleavage events

Abbreviations: Leda-1, liver endothelial differentiation-associated protein-1; Pianp, PILR alpha associated neural protein; PILR, paired immunoglobin-like type 2 receptor; AJAP-1, adherens-junction-associated protein-1; MMPs, matrix metalloproteinases; ADAM, A disintegrin and metalloproteinase; PS, presenilin; CTF, C-terminal fragment; ICD, intracellular domain; WT, wild type; aa, amino acids; kDa, kilodalton.

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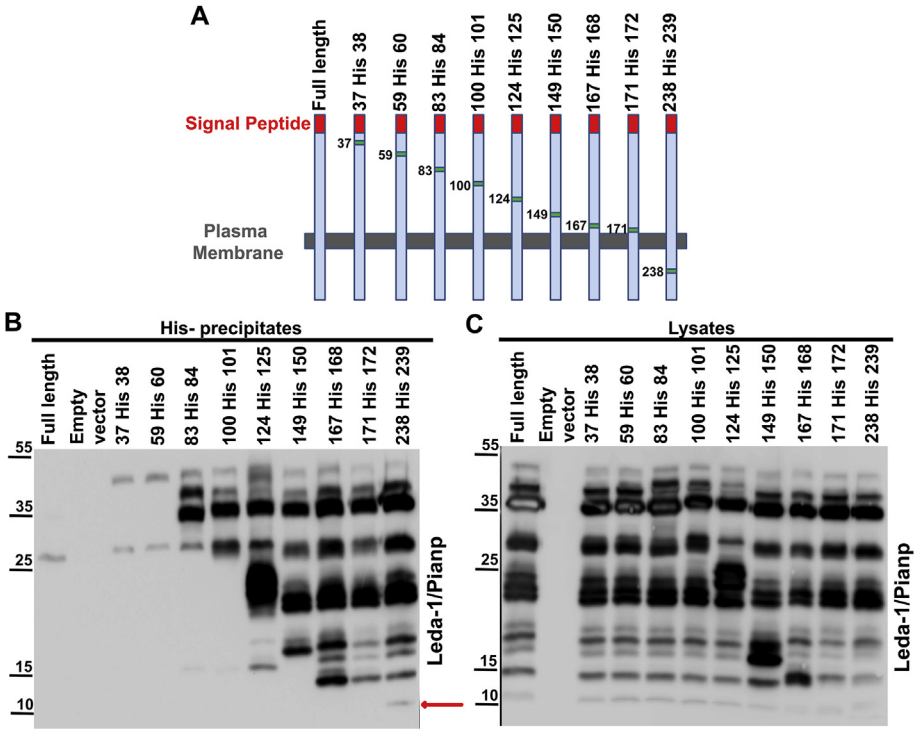

Fig. 1. Comprehensive characterization of cleavage events during the processing of Leda-1/Pianp. (A) Schematic representation of the generated variants of Leda-1/Pianp. The positions of the inserted 6x-His-tags are indicated by green bars. (B) WB of Leda-1/Pianp of anti-His precipitates from CHO cells transfected with the indicated variants of Leda-1/Pianp. The red arrow indicates a fragment that is only visible in variant 238His239. (C) WB of Leda-1/Pianp of lysates from CHO cells transfected with the indicated variants of Leda-1/Pianp.

and the involved proteases during constitutive posttranslational processing of Leda-1/Pianp have not been delineated in detail. Therefore, it was our goal to comprehensively characterize proteolytic processing of Leda-1/Pianp.

2. Materials and methods

2.1. Reagents

Following reagents were used – DMSO, PMA, APMA (Sigma), TAPI-1, γ-secretase inhibitor IX, Lactacystin (Calbiochem), GM6001 (US Biological).

2.2. Cloning

Mouse Leda-1/Pianp full length cDNA IRAVp968A09128D (IMAGE ID 6413408) was prepared by PCR reactions using the following primers – MmLeda1_NheI (forward) 5′-ACTGCTAGCAGCCGCCACCATGTGGTCTGCTCAACTGCTGTCC-3′, MmLeda1_BstBI (reverse) 5′-GTTGTTCGAAGCATCACAGATTCACCAGGGGA-

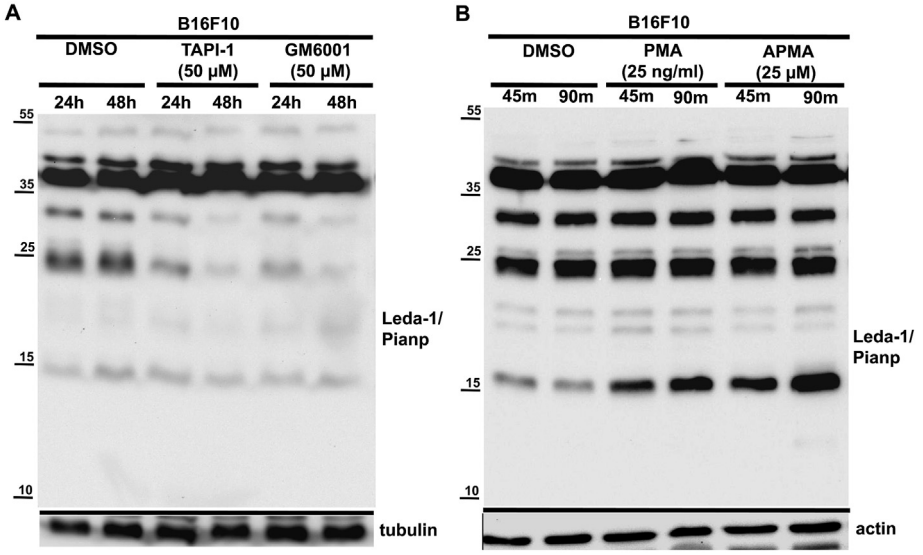

Fig. 2. Leda-1/Pianp is constitutively and inducibly cleaved by MMPs. (A) WB of Leda-1/Pianp and house-keeping gene tubulin of B16F10 cells treated with DMSO (control), TAPI-1 and GM6001 for 24 h and 48 h (B) WBof Leda-1/Pianp and house-keeping gene b-actin of B16F10 cells treated with DMSO (control), PMA and APMA for 45 min and 90 min.