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Cellular characterization and regulation of type-I transmembrane protein Leda-1/Pianp in vitro and behavioral phenotyping of Leda-1/Pianp$^{-/-}$ mice

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Leda-1/Pianp was initially identified as a hepatic microenvironment dependent gene expressed by rat LSEC. ClustalX alignment of rat, mouse and human Leda-1/Pianp protein sequences revealed 97% similarity in amino acid sequence between them. Enzymatic deglycosylation of Leda-1/Pianp led to shift of the band size from ~36-38 kDa to ~26 kDa. Therefore, Leda-1/Pianp is a glycoprotein. The only known homolog to Leda-1/Pianp is AJAP-1. Loss of AJAP-1 was found to be associated with increased glioma cell proliferation and migration. Transcripts analysis of Leda-1/Pianp in human and mouse revealed that only neural tissues in both were abundantly expressing mRNA. Western blotting from rat and mouse brain lysates also confirmed expression of Leda-1/Pianp in brain. Further experiments identified Leda-1/Pianp as a ligand for PILR□. However, comprehensive knowledge of Leda-1/Pianp expression, proteolytic processing/ posttranslational modifications and functions in vitro and in vivo are still lacking. Therefore this doctoral thesis aims at characterizing expression, posttranslational modifications and cellular and physiological functions of Leda-1/Pianp in vivo and in vitro.

Human astrocytes, glioblastoma and BALB/c but not C57BL6/J lymphoid organs (lymph node, spleen and thymus) were expressing Leda-1/Pianp. It could be shown that Leda-1/Pianp is cleaved extracellularly by proprotein convertase Furin and the cleavage takes place before Leda-1/Pianp is transferred to the surface plasma membrane. Leda-1/Pianp is further cleaved by sheddases (ADAM10 and 17) extracellularly and □-secretase intramembraneouly. Upon LPS stimulation in RAW 264.7 cells Leda-1/Pianp is degraded proteolitically by MMPs (MMP9 and/or 13). The ligand-receptor pair of Leda-1/Pianp and PILR□ is contrarily regulated in RAW 264.7 cells upon LPS stimulation indicating that this counter regulation is involved in the LPS response of myeloid cells. Functional analysis of Leda-1/Pianp in MEF cells revealed that Leda-1/Pianp can influence migration and adhesion but not proliferation. Leda-1/Pianp$^{-/-}$ mice were viable and lab values of plasma were comparable to wild type mice. Isolation and western blotting of several mouse brain regions revealed that Leda-1/Pianp is expressed in all the major brain regions. Baseline parameters such as locomotor activity, rearings, and body weight were also similar between Leda-1/Pianp$^{-/-}$ and wild type mice. Therefore, a general, emotional behavioral test and known endophenotypes of psychiatric diseases were tested. These tests revealed that Leda-1/Pianp$^{-/-}$ mice had anxiety, depression and recalling context related memory function disorder. Tests specific for known endophenotypes of psychiatric diseases also gave hints for ASD or schizophrenia in Leda-1/Pianp$^{-/-}$ mice.

In conclusion, it was shown here that Leda-1/Pianp is proteolytically cleaved by Furin, sheddasses, MMPs and □-sectretase during posttranslational modification. When stimulated with LPS, the ligand-receptor pair of Leda-1/Pianp and PILR□ is contrarily regulated in myeloid cells. Leda-1/Pianp$^{-/-}$ mice had anxiety-like behavior, higher stress coping ability, deficit in contextual learning and sociability, and hyper-locomotion activity upon psychostimulant administration which are the symptoms of several neurological diseases such as ASD, dementia and schizophrenia. Neurotransmitter analyses, fMRI and additional behavioral studies are necessary to gain more insight into this behavioral and its underlying molecular mechanisms.