Journal Club

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# Relevance of the Proteolytic Processing of Reelin by ADAMTS-3 in Brain Functions

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Review of Ogino et al.

Reelin is a prominent extracellular glycoprotein that has roles in the CNS throughout development and maturity. During development, Reelin is produced by Cajal-Retzius cells at the marginal zone above the cortical plate and by granule cells in the cerebellum, and it controls cytoskeletal dynamics required for the laminar migration of neurons, and remodeling of dendrites and spines. In the mature brain, GABAergic interneurons and glutamatergic pyramidal neurons in layer II of the entorhinal cortex take over the production of Reelin, which continues to regulate neuronal function and synaptic activity (Knuesel et al., 2009). During aging, reduction of Reelin expression contributes to synaptic plasticity defects and cognitive impairments (Doehner and Knuesel, 2010). A decrease in Reelin activity also contributes to pathogenesis and/or deterioration in several neuropsychiatric and neurodegenerative disorders, including schizophrenia, frontotemporal dementia, and Alzheimer's disease. Therefore, developing therapeutic strategies to increase Reelin activity might restore brain functions in these pathological conditions.

Reelin binds to the apolipoprotein E receptor 2 and the very low-density lipoprotein receptor, and this triggers phosphorylation of the cytosolic adaptor protein disabled-1 (Dab-1) by kinases of the Src family, which promotes the activation of downstream signaling. Reelin-Dab-1 signaling leads to the phosphorylation and subsequent inactivation of glycogen synthase kinase-3$\beta$. Ultimately, the decrease of glycogen synthase kinase-3$\beta$ activity prevents excessive phosphorylation of the microtubule-associated protein Tau, thereby preserving neuronal integrity, axonal transport, and synaptic plasticity (Doehner and Knuesel, 2010; Guo et al., 2017). Phosphorylated Dab-1 is then degraded via the ubiquitin-proteasome pathway, which ensures a fine-tuning of the Reelin response (Arnaud et al., 2003).

Full-length Reelin is composed of an N-terminal region (NTR), eight Reelin repeats (RRs), and a C-terminal region (CTR). The central region (RR3-RR6) is required for receptor binding, and the NTR and CTR are responsible for Reelin multimerization and folding, respectively. After secretion in the extracellular space, Reelin is subject to specific proteolytic cleavage at RR3 (Pro1244-Ala1245) (N-terminal cleavage) and/or between RR6 and RR7 (Ala2688-Asp2689) (C-terminal cleavage). This can generate five fragments depending on the protease involved: NR2 (from NTR to RR2) and R78C (from RR7 to the CTR) fragments, but also three fragments containing the receptor binding region: for instance, NR6 (from NTR to RR6), R36 (from RR3 to RR6), and R38C (from RR3 to the CTR) fragments (Koie et al., 2014; Sato et al., 2016). Which protease is involved in Reelin cleavage and how cleavage at these sites affects Reelin function remains elusive. Whereas some reports indicate that the N-terminal cleavage of Reelin promotes the conversion of "inactive" full-length Reelin into an "active" fragment (Jossin et al., 2007; Tinnes et al., 2013), a growing body of evidence also shows that the N-terminal cleavage of Reelin negatively modulates Reelin function in neuron cultures (Kohno et al., 2009; Koie et al., 2014; Ogino et al., 2017). In a study recently published in *The Journal of Neuroscience*, Ogino et al. (2017) identified a key protease that cleaves Reelin, and they show that cleavage by this protease inactivates Reelin.

By using chromatography and mass spectrometry, Ogino et al. (2017) discovered that N-terminal cleavage of Reelin in primary cultures of neurons was achieved by the metalloproteinase ADAMTS-3 (type 3 a disintegrin and metalloproteinase with thrombospondin motifs). This result was confirmed by the abolishment of the N-terminal cleavage in the culture media of ADAMTS-3 knock-out (KO) neurons. Importantly, the authors showed that the expression of ADAMTS-3 in mouse em-

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bryos and adults greatly overlaps with the expression of neurons targeted by Reelin. For instance, hybridization revealed the presence of ADAMTS-3 in TBR1-positive neurons present in the deep cortical plate as well as in upper cortical layers. In adults, the expression of ADAMTS-3 was widespread throughout the brain, including in the cerebellum and in the cortex. Thus, ADAMTS-3 is expressed in regions where it may process Reelin *in vivo*.

Ogino et al. (2017) next looked at the expression of Reelin and its intracellular effectors in the cerebral cortex of wild-type and ADAMTS-3 KO mice during embryonic development. At embryonic day 13.5 and 16.5, levels of NR6 were increased, whereas levels of NR2 were decreased. This apparent reduction of the N-terminal cleavage of Reelin in ADAMTS-3 KO mice increased activation of Reelin-Dab-1 signaling as indicated by Dab-1 degradation and decreased levels of phosphorylated Tau in these mice. Surprisingly, these changes in ADAMTS-3 KO mice did not alter the positioning of radially migrating neurons, suggesting that the NTR is not required for the laminar migration of neurons. Nevertheless, ~25%–40% of NR2 fragments were still present in the cortex of ADAMTS-3 KO embryos, suggesting that (1) ADAMTS-3 KO mice might show compensatory upregulation of other proteases that cleave Reelin, (2) ADAMTS-3 might not be the only protease cleaving Reelin *in vivo*, or (3) another protease controls the laminar migration of neurons ensured by Reelin during embryogenesis.

To study the role of ADAMTS-3 postnatally, Ogino et al. (2017) conditionally knocked out ADAMTS-3 (cKO) selectively in cortical and hippocampal excitatory neurons. Similar to their observations during embryonic development, they found decreased expression of the Reelin NR2 fragment and Dab-1 in the postnatal cortex and hippocampus of ADAMTS-3 cKO mice at P7 and P28 (Ogino et al., 2017). The activation of Reelin-Dab-1 signaling and/or the decrease of Reelin NR2 fragment promoted dendrite elongation and dendritic branch formation in excitatory neurons (Ogino et al., 2017). This result is in line with several studies reporting the dendritic remodeling of excitatory neurons by Reelin (Niu et al., 2008; Chameau et al., 2009). Considering the importance of spine modeling by Reelin and its impact on LTP in the prefrontal cortex of juvenile mice (Iafrati et al., 2014), it would have been valuable to look at dendritic spine density in ADAMTS-3 cKO mice.

The proteolysis of Reelin by ADAMTS-3 might be a decisive upstream molecular mechanism leading to Reelin inactivation, which may contribute to and/or worsen the pathogenesis of neuropsychiatric and neurodegenerative disorders. Previous studies have shown that several proteases, including tissue plasminogen activator, meprin $\alpha$ and $\beta$, matrix metalloproteinase-9, and ADAMTS-4 and -5 can cleave Reelin (Krstic et al., 2012; Lussier et al., 2016; Sato et al., 2016), but ADAMTS-3 KO mice are the only KO mice in which altered levels of Reelin have been documented. Thus, abnormal activation of ADAMTS-3-Reelin signaling might prevent spine development and LTP in the postnatal brain and contribute to the development and/or deterioration of neuropsychiatric disorders, such as schizophrenia. It may also promote Tau hyperphosphorylation and aggregation, and the subsequent formation of neurofibrillary tangles in Alzheimer's disease (Guo et al., 2017). Therefore, the development of monoclonal antibodies or specific inhibitors targeting ADAMTS-3 may lead to innovative therapeutic strategies to prevent ADAMTS-3-dependent processing of Reelin and reduce synaptic plasticity defects and cognitive impairments in neuropsychiatric and neurodegenerative diseases.

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