PERSPECTIVE

Intracellular sorting pathways of the amyloid precursor protein provide novel neuroprotective strategies

Alzheimer's disease (AD) is the most common cause of senile dementia. It is characterized by the formation of plaques mainly composed of the amyloid-beta peptide (Aβ). Diverse lines of evidence support the notion that accumulation of Aβ is a primary cause of AD pathogenesis (Huang and Mucke, 2012).

Amyloid precusor protein (APP) processing is dependent on its subcelluar trafficking pathway: Aβ is derived from APP by proteolytic processing. APP is a type I transmembrane protein with a large extracellular moiety and a short cytoplasmic domain. Its physiological function is still incompletely understood. APP can be processed along two main pathways. Shedding by the β-secretase releases a large soluble N-terminal fragment (sAPPβ) initiates the amyloidogenic pathway. The remaining C-terminal membrane anchored portion is subsequently cleaved within its transmembrane domain by the γ-secretase complex releasing Aβ. In the alternative non-amyloidogenic pathway, APP is first shed by α-secretase releasing a longer soluble N-terminal fragment (sAPPα). Cleavage by α-secretase occurs within the Aβ-domain. This precludes Aβ generation by subsequent processing of the remaining C-terminal transmembrane fragment by the γ-secretase complex (Huang and Mucke, 2012).

Different subcellular localizations of each of these secretase activities have been observed (Small and Gandy, 2006). APP is cleaved by α-secretase predominantly in secretory vesicles and at the cell surface whereas β-secretase and γ-secretase activities are mainly found in endosomal compartments (**Figure 1**). Thus, amyloidogenic and non-amyloidogenic processing of APP is highly dependent on its intracellular itinerary. Accordingly, altered subcellular targeting of APP directly affects the degree to which Aβ is generated. Therefore, understanding the molecular machinery underlying the subcellular targeting of APP is key to identify neuroprotective tools that modulate APP trafficking. Directing APP to subcellular compartments harboring predominantly α-secretase would be neuroprotective by reducing Aβ generation and thereby ameliorate AD symptoms.

SorLA modulates APP transport and processing: Genetic analyses strongly support the role of APP and the γ-secretase complex in AD because the majority of familial early onset AD cases result from mutations in the genes encoding APP or the γ-secretase complex subunit presenilin. These genes are usually not effected in the late-onset form of AD that accounts for more than 90% of all cases. Numerous genome wide association studies aimed at identifying risk factors for the late-onset form of AD and the sorting receptor SorLA was among the candidate genes. Genetic analyses suggest that SorLA modulates the risk for late-onset as well as for early-onset AD (Thakurta and Andersen, 2015). Moreover, SorLA expression levels are reduced in brains of AD patients as compared to healthy non-demented subjects. SorLA (also termed LR11 or SORL1) is a mosaic receptor of the Vps10p-Domain receptor family. The family comprises the five type I transmembrane proteins SorLA, Sortilin, SorCS1, SorCS2, and SorCS3 (Hermey, 2009). SorLA is the only Vps10p-Domain receptor sharing structural similarities with low-density lipoprotein receptors and it interacts directly with APP. The interaction has been mapped to the complement type repeat (CR) domains of SorLA and the carbohydrate (E2) domain of APP (Mehmedbasic et al., 2015). SorLA expression reduces APP amyloidogenic processing and an inverse correlation between SorLA expression and Aβ generation has been observed (Willnow and Andersen, 2013). SorLA acts as a retrograde sorting receptor for APP and conveys APP retention in the trans-Golgi network (TGN). Biochemical analysis demonstrated that the SorLA-APP interaction is favored at low pH (Mehmedbasic et al., 2015). This is in agreement with the notion that the interaction occurs in endocytic compartments characterized by an acidic pH and SorLA conveys retrograde transport of APP away from endosomes (**Figure 1**). Cytosolic adaptor proteins mediating transport between late endosomal compartments and the TGN have been shown to interact with SorLA. These comprise GGAs, PACS, AP-1, and retromer. The latter is a protein complex that plays a role in regulating APP processing and has been linked to AD. SorLA seems to act as a functional bridge between retromer and APP. Recently, the retromer has been the target of pharmacological interventions by introducing a small molecular chaperone that decreases amyloidogenic APP processing by stabilizing the retromer complex (Mecozzi et al., 2014). This study demonstrated the neuroprotective capacity of modulators of the APP trafficking pathway.

The role of SorCS1 in AD: Genetic studies have linked another member of the Vps10p-Domain receptor family, SorCS1, to the late-onset form of AD (Reitz et al., 2011). SorCS1 has been localized to the nervous system, notably to the cerebral cortex and hippocampus (Oetjen et al., 2014), but transcripts have been detected as well outside of the nervous system. The receptor is expressed in different splice variants encoding identical extracellular and transmembrane moieties, but different cytoplasmic domains (Hermey, 2009). The splice variants convey different subcellular localizations and cellular functions. Some variants show a high abundance on the cellular surface und a low internalization capacity, whereas others present a low surface expression, are localized mainly to the Golgi and endosomal compartments, and have a high internalization capacity. The adaptor-protein 2 (AP-2) complex mediates the internalization of these variants and the receptors are capable of targeting internalized cargo to lysosomes (Nielsen et al., 2008). Experimental evidence support that SorCS1 variants are not engaged in Golgi-endosomal transport (Nielsen et al., 2008). Moreover, a direct interaction with adaptor proteins mediating Golgi-endosomal transport *e.g.*, GGAs has not been demonstrated although it has

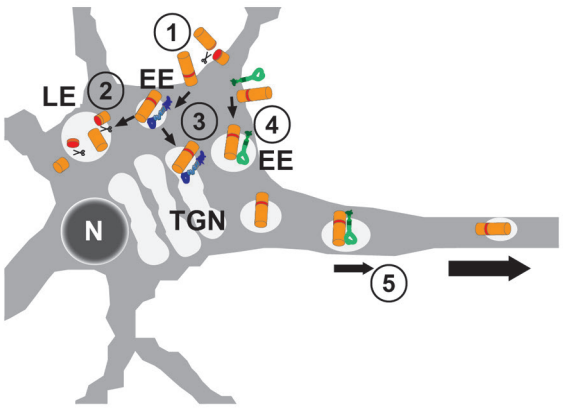

Figure 1 Intracellular itinerary and processing of the amyloid precursor protein (APP).
Altered APP trafficking is thought to modulate its processing. APP (orange) is targeted through the secretory pathway to the cellular surface. Here, non-amyloidogenic processing by α-secretase occurs ① or APP is internalized to early endosomes (EE). On its passage through endosomal compartments to late endosomes amyloidogenic processing of APP by β-secretase and γ-secretase results in Aβ (red) ② . An alternative pathway targets APP back to the trans-Golgi network (TGN) and prevents amyloidogenic processing ③ . SorLA (blue) acts as a retention factor and targets APP from endosomes to the TGN. SorCS1 does not tie APP to the cellular surface. SorCS1c (green) is internalized and co-transported with APP and shares a common endocytic pathway but does not modulate its endocytic transport ④ . Co-expression of SorCS1c reduces anterograde APP transport ⑤ suggesting that SorCS1c can retain APP from insertion into anterograde transport vesicles. EE: Early endosomes; LE: late endosomes; N: nucleus; TGN: trans-Golgi network.

been carefully studied.

Hippocampal expression of SorCS1 is transiently induced by neuronal plasticity inducing stimuli and it may play a role in plasticity-related events in the nervous system (Hermey, 2009). This notion is supported by a recent study demonstrating an interaction of SorCS1 with AMPA receptors and neurexins and modulation of their subcellular trafficking by SorCS1 (Savas et al., 2015). Moreover, SorCS1 is differentially processed at its N-terminus by proprotein convertases and serves as a substrate of α- and γ-secretase (Hermey, 2009). Interaction of SorCS1 and APP has been demonstrated by co-immunoprecipitations (Lane et al., 2010; Reitz et al., 2011) and observed in proteome analysis (Savas et al., 2015). SorCS1 expression levels are reduced in the amygdala from AD patients as compared to unaffected subjects (Reitz et al., 2011). Studies using transfected cells and knock-out mice suggest that high SorCS1 expression levels result in a modest decrease of Aβ levels, and reduction of SorCS1 expression results in an increase of Aβ (Lane et al., 2010; Reitz et al., 2011). This inverse relation between SorCS1 expression and Aβ levels resembles the implications of altered SorLA expression on Aβ levels. Accordingly, a role of SorCS1 in APP transport reminiscent to that of SorLA has been suggested. This idea has been further supported by co-immunoprecipitations of SorCS1 with SorLA and the retromer subunit Vps35. However, a direct binding of SorCS1 to the retromer complex or a retromer-mediated transport of SorCS1 awaits demonstration. The notion that SorCS1 and SorLA play an almost identical role in APP trafficking is further opposed by the previous finding that in contrast to SorLA none of the SorCS1 splice variants is implicated in endosome-TGN shuttling (Nielsen et al., 2008). Therefore, it is likely that SorCS1 plays an alternative role in APP trafficking.

We analyzed if SorCS1 modulates internalization and intracellular transport of APP (Hermey et al., 2015). We focused on two splice variants of SorCS1 that can be regarded as prototypes. SorCS1b has a high surface expression and low internalization capacity and SorCS1c has a low surface expression and high internalization capacity. We generated fluorophore tagged variants of both receptors harboring a Venus-tag between the extracellular and transmembrane domains to ensure N-terminal processing and interaction with cytosolic adaptor proteins. We found that both receptor constructs are correctly targeted and the SorCS1c variant is internalized. We confirmed co-localization and co-immunoprecipitation of APP and both tagged SorCS1 variants.

The endocytic uptake of APP is a key step in its amyloidogenic processing. Assuming an interaction of SorCS1 and APP at the cellular surface we analyzed if the endocytic variant SorCS1c promotes uptake of APP or if the non-internalizing isoform SorCS1b ties APP to the cellular surface. We observed that internalization of APP is independent of SorCS1 and our data suggest a weak interaction of both proteins on the plasma membrane. However, SorCS1c and APP are internalized with similar transport characteristics through a common endocytic pathway. Both are transported *via* Rab5-positive early endosomes to late endosomal compartments. We analyzed as well vesicular co-transport of APP and SorCS1 in axons of dissociated primary cortical neurons in which microtubules have uniform polarity. Time-lapse imaging revealed that vesicles with similar transport characteristics are positive for SorCS1c and SorCS1b. Notably, the amount of SorCS1c transport vesicles was higher than the amount of SorCS1b transport vesicles. This is in agreement with the higher surface expression of SorCS1b. However, both variants showed a median transport velocity of about 1 μm/s. This suggests transport of both receptor variants by the same fast transport machinery, most likely by kinesin. APP was co-transported with SorCS1b and SorCS1c. The co-expression of SorCS1b with APP did not cause a significant alteration of APP transport. In contrast, we observed after co-expression of SorCS1c a significant reduction of the relative amount of APP anterograde transport vesicles and a corresponding increase of the number of stationary vesicles. These data support the notion that SorCS1 functions as a sorting receptor for APP. The observation that SorCS1c, but not SorCS1b alters the transport characteristics of APP is in agreement with the concept that differences in the cytoplasmic domains determine distinct functions of SorCS1 variants.

We did not observe a significant change in APP retrograde transport after co-expression of APP and SorCS1c. However, SorCS1c retains APP from anterograde transport vesicles and increases the fraction of APP localized to stationary vesicles (**Figure 1**). Future studies will be needed to clarify if these changes are accompanied by altered subcellular targeting of APP in neurons. Additional studies will have to include as well detailed analysis of APP transport in neurons under SorCS1 knockout conditions.

The current analyses suggest a model in which SorCS1 is not regulating Golgi-endosomal trafficking of APP, but is regulating its sorting and anterograde transport. Small alterations in APP trafficking may have a modest impact on Aβ generation, but probably result as a long-term effect over a lifetime in dramatic Aβ accumulation. Therefore, understanding the intracellular sorting pathways of APP and its determinants in more detail will underlie the development of novel neuroprotective strategies.

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