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Featured Article

SORLA regulates calpain-dependent degradation of synapsin

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Abstract

Introduction: Sorting-related receptor with A-type repeats (SORLA) is an intracellular sorting receptor in neurons and a major risk factor for Alzheimer disease.
Methods: Here, we performed global proteome analyses in the brain of SORLA-deficient mice followed by biochemical and histopathologic studies to identify novel neuronal pathways affected by receptor dysfunction.
Results: We demonstrate that the lack of SORLA results in accumulation of phosphorylated synapsins in cortex and hippocampus. We propose an underlying molecular mechanism by demonstrating that SORLA interacts with phosphorylated synapsins through 14-3-3 adaptor proteins to deliver synapsins to calpain-mediated proteolytic degradation.
Discussion: Our results suggest a novel function for SORLA which is in control of synapsin degradation, potentially impacting on synaptic vesicle endocytosis and/or exocytosis.

Keywords: Alzheimer's disease; Risk factor; Sporadic AD; SORLA; *Sorl1*; Synapse

1. Introduction

Sorting-related receptor with A-type repeats (SORLA, also known as LR11) is a member of the VPS10P domain receptor gene family, a unique class of type 1 membrane proteins expressed in the mammalian nervous system [1,2]. Initially, these orphan receptors were recognized by their structural similarity to the vacuolar protein sorting 10 protein (VPS10P), a trafficking receptor in yeast that sorts target proteins between golgi and endosomal compartments [3]. However, recently, VPS10P domain receptors emerged as central regulators of neuronal viability and function implicated in many diseases of the nervous system including frontotemporal lobar degeneration [4], Alzheimer disease (AD) [5,6], and bipolar disorders [7].

SORLA is best known for its activity as sorting receptor for the amyloid precursor protein (APP), the main etiologic agent in AD. According to current concepts, SORLA inhibits trafficking of APP into cellular compartments where secretases reside, thereby reducing the extent of proteolytic breakdown of this precursor into neurotoxic amyloid-β peptides. Based on cumulative evidence from studies in cultured cells [5,8,9] in animal models [2,10] and in humans [6], SORLA is now considered a major risk factor for the sporadic form of AD (reviewed in [11]).

Although its contribution to amyloidogenic processes is well appreciated, there are reasons to believe that the function of SORLA in the nervous system may not be restricted to the control of APP transport and processing. Thus, transcription of *Sorl1* (the gene encoding SORLA) in neurons is strongly induced by brain-derived neurotrophic factor (BDNF), implicating this receptor in neurotrophin-dependent pathways [12].

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To elucidate hitherto unknown functions for SORLA in the nervous system, we used an unbiased proteomics approach to compare the brain proteome of SORLA deficient and control mice. Our studies uncovered upregulation of several phosphorylated (active) forms of synapsin 1 and 2 as a consequence of receptor dysfunction. Using biochemical and histopathologic studies, we show that SORLA physically interacts with phospho-synapsins and promotes the turnover of these synaptic vesicle (SV)–associated proteins by the protease calpain. Our results suggest a novel function for SORLA as modulator of neuronal endocytosis and/or exocytosis.

2. Results

2.1. Proteins related to synaptic endocytosis and/or exocytosis are altered in the brain of SORLA-deficient mice

We used a two-dimensional polyacrylamid gel electrophoresis (2D-PAGE) approach to compare the cortical proteome of 1-month old SORLA-deficient and control mice. 2D-PAGE analysis documented 94 significantly altered protein spots in *Sorl1*$^{-/-}$ compared to *Sorl1*$^{+/+}$ cortices (Student's t-test, $P < .05$; n = 6 biological replicates). Subsequent analysis by mass spectrometry revealed the identity of 53 of these spots (Supplementary Table 1A). A representative gel image of mouse cortex is shown in Supplementary Fig. 1. Enrichment analysis of the data set compared to the whole mouse genome data set or to a data set of murine brain proteins present in 2D-PAGE [13] identified the gene ontology term "synaptic transmission" —among other functions—as being significantly enriched in this set of altered proteins ($P = 6.57 \times 10^{-6}$ and $P = 2.20 \times 10^{-3}$, respectively). The full result of the enrichment analysis is shown in Supplementary Fig. 2.

We also performed 2D-PAGE analysis on hippocampal extracts. As in cortex, we found predominant alterations in proteins related to synaptic endocytosis and/or exocytosis. Altogether, 90 protein spots were significantly altered in *Sorl1*$^{-/-}$ compared to *Sorl1*$^{+/+}$ hippocampi (Student's t-test, $P < .05$; n = 6 biological replicates). Of these, 58 spots were identified by mass spectrometry (Supplementary Table 1B). Analysis revealed enrichment of the gene ontology term "transmission of nerve impulse" when compared to the whole mouse genome data set or to the data set of murine brain proteins in 2D-PAGE [13] ($P = 6.00 \times 10^{-4}$ and $P = 7.30 \times 10^{-3}$, respectively, Supplementary Fig. 2).

When comparing our two data sets, we identified two protein families that were changed in expression in both, hippocampus and cortex, of *Sorl1*$^{-/-}$ mice, indicating a more direct functional connection to SORLA. These two protein families were synapsins and 14-3-3 adaptor proteins, both of which are related to synaptic endocytosis and/or exocytosis.

2.2. Phosphorylated synapsins accumulate in the brain of SORLA-deficient mice

Several protein spots representing modified forms of synapsins 1 and 2 were significantly increased in abundance in cortex and hippocampus of *Sorl1*$^{-/-}$ animals (Supplementary Table 1; Fig. 1A). Quantification of the respective spot volumes documented an approximate ratio of 1.5 to 1.8 in cortex of receptor deficient compared to control animals (Fig. 1B; $P < .05$). Similar increases were seen for synapsin 1 (ratio 1.7; $P = .01$) and synapsin 2 (ratio 1.3; $P = .02$) in hippocampus (Supplementary Table 1B).

Synapsins are phosphoproteins with eight phosphorylation sites functionally characterized so far. Typically, phosphorylation induces mobilization of SV that is sequestered by synapsins otherwise (reviewed in [14]). Because accumulation of synapsins was seen for multiple isospots in our 2D gels, indicative of alternative post-translational modification, we tested the phosphorylation status of these synapsin isoforms using NanoLC-ESI-MS/MS mass spectrometry. Mass spectrometry revealed that all isoforms of synapsin 1 and 2 accumulating in *Sorl1*$^{-/-}$ cortex were phosphorylated (Fig. 1B, Supplementary Table 2). In most cases, synapsins were phosphorylated at site 1 (Ser9 in synapsin 1, Ser10 in synapsin 2), a site phosphorylated on depolarization after Ca^{2+} influx. Other identified phosphorylations encompassed sites 4, 5, 6, and 7 [14]. Thus, of eight functionally characterized phosphorylation sites in synapsins, six were present in the protein spots accumulating in the SORLA-deficient cortex. Additional phosphorylations, which had been identified but not functionally characterized thus far (http://www.uniprot.org/), were also present in the accumulating synapsin 1 and 2 spots (Fig. 1B, Supplementary Table 2).

To substantiate the accumulation of phosphorylated (active) forms of synapsin 1 and 2 in receptor-deficient brains, we analyzed SORLA-deficient mouse cortices by 2D-Western blotting applying antibodies that specifically recognize synapsins phosphorylated at site 1, site 4, or site 6. In line with our proteome data (Fig. 1B), synapsin 1 phosphorylated at sites 1, 4, and 6 and synapsin 2 phosphorylated at site 1 were significantly increased in abundance in mutant mice (Fig. 2; n = 4–6, unpaired Student's t-test). Taken together, accumulation of multiphosphorylated forms of synapsin 1 and 2 was identified in the *Sorl1*$^{-/-}$ brain, indicating a status of chronic activation of synapsins 1 and 2 as a consequence of SORLA deficiency.

2.3. SORLA interacts with synapsin via 14-3-3-adaptor proteins

Next to synapsins, 2D-PAGE proteome analysis also uncovered significant changes in expression levels of 14-3-3 proteins that were reduced in both cortex and hippocampus of SORLA deficient as compared to control mice (Supplementary Table 1). For cortex, the spot volume ratio