Intersectin 1: a molecular linker in the central nervous system

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Abstract: Down syndrome (DS) is the most common cause of cognitive impairment associated with a congenital chromosomal abnormality, trisomy of chromosome 21. Mental retardation and congenital heart defects are key features of DS. All DS individuals develop early-onset Alzheimer's disease-like neuropathology. Intersectin 1 gene is localized on human chromosome 21, the critical region of DS, and it has higher expression in the brain of DS patients than in normal individuals. So fully understanding functions of intersectin 1 is critical for revealing the pathogenesis of DS. Intersectin 1 protein has two isoforms: intersectin 1-L and intersectin 1-S. This review will focus on the distribution, expression characters and functions of intersectin 1 in the central nervous system.

Keywords: intersectin 1; neuron; endocytosis; exocytosis

1 Introduction

Intersectin 1 is a highly conserved protein during evolution and possesses multiple domains. It is also called ESH1, Dap160, ITSN, or ESE-1^[1]. Intersectin 1 protein has two isoforms: the long isoform (intersectin 1-L) and the short isoform (intersectin 1-S). Intersectin 1-S isoform contains two EH domains, a coiled-coil KLERQ domain, and five SH3 domains. Intersectin 1-L contains DH domain, PH domain and C2 domain in addition to the domains shared with intersectin 1-S^[2] (Fig. 1). It has the ability to bind protein-rich polypeptide and form complex with dynamin and synaptosomal-associated protein-23/25 (SNAP-23/25), which are important proteins for endocytosis and exocytosis^[3]. The EH domain of intersectin 1 has been reported to interact with endocytic proteins, including secretory carrier membrane protein 1 (SCAMP1) and epsins/Ibpsi^[4]. The coiled-coil KLERQ domain has been shown to bind Eps15 and SNAP25^[2]. The SH3 domain can interact with several proteins essential for

Fig. 1 Domain structure of two isoforms of intersectin 1. The top is the intersectin 1-L isoform, and the bottom is intersectin 1-S isoform.

endocytosis including dynamin, neural Wiskott-Aldrich syndrome protein (N-WASP), synaptojanin, and so on^[5-8]. The DH domain serves as a guanine nucleotide exchange factor (GEF) for Rho protein family, which includes Rho, Rac and Cdc42^[9]. The PH domain can interact with lipid and cell membrane directly in coordination with the DH domain^[10]. The C2 domain binds phospholipid membrane, protein or other soluble inositol polyphosphate through both Ca^{2+} -dependent and independent mechanisms^[8].

Intersectin 1 is highly related with neuronal degenerative diseases such as DS, Alzheimer's disease (AD) and Huntington disease. DS patients usually have early incidence of AD by their mid 40s, presenting the classical pathological markers of β -amyloid plaque deposition and neurofibrillary tangles. Enlarged early endosomes were reported as the earliest neuropathologic lesion in sporadic AD. In DS, early endosomes are markedly enlarged in some pyramidal neu-

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rons as early as 28 weeks of gestation, decades before classical AD neuropathology develops. Whereas early endosomes are the site of β -amyloid (A β) peptide generation and internalization and initial processing of the proteins involved in AD, including the β protein precursor (β PP) and apolipoprotein E (ApoE)^[11]. It has been found that mutation of *Itsn1* is involved in damaged vesicle trafficking and endocytic function, implying a role for intersectin 1 in the early endocytic anomalies reported in DS brains, which may have ramifications of the onset of AD^[12]. Some researches elucidated that intersectin 1 promoted polyglutamine (polyQ)-mediated neurotoxicity. Intersectin 1 can enhance mutant huntingtin aggregation through activation of the c-Jun-NH2-terminal kinase (JNK)-MAPK pathway. There is a reciprocal

relationship between intersectin 1 and huntingtin: intersectin 1 enhances huntingtin aggregation and toxicity; conversely, huntingtin inhibits the cooperativity between intersectin 1 and the epidermal growth factor receptor (EGFR) signaling transduction pathway^[13].

2 Distribution in the central nervous system

Our previous studies reported that the cellular distribution of two isoforms intersectin 1-L and intersectin 1-S had their own specificity in the central nervous system. We found that intersectin 1-L was highly enriched in neurons; while intersectin 1-S was mainly detected in astrocytes and microglia, and little intersectin 1-L was found in glia cells (Fig. 2C)^[5]. These results suggest that the expressions of intersectin 1-L

Fig. 2 Expression of intersectin 1 in brain tissues and separate cell types in the central nervous system. A and A': Neurons of the central amygdaloid nucleus showed perinuclear enrichment of intersectin 1 immunoreactivity (arrows). B and B': Neurons of the globus pallidus with intensely immunostained cell bodies (large arrows) and fibers (small arrows). Scale bar, 80 μ m in A and B; 20 μ m in A' and B'. (Ma *et al.*, J Neurosci Res, 2003^[5])

and intersectin 1-S are strictly regulated in different cell types, and their unique cellular distribution should correspond to their functions. Our immunohistochemistry results also reveal that intersectin 1 has widespread distribution throughout the brain. Particularly intense expression was observed in the somatodendritic regions of cortex neurons, hippocampus, globus pallidus, subthalamic nucleus, and substantia nigra (Fig. 2A, B)^[5]. For the two isoforms of intersectin 1, intersectin 1-S has been reported to be found in many other cell types including endocrine tissues of the pancreas, adrenal, thyroid and pituitary; while intersectin 1-L is mainly expressed in the brain^[14].

3 Cellular functions

3.1 Endocytosis and exocytosis Intersectin 1 is regarded as an adaptor protein, according that it has the ability to bind different kinds of proteins and can interact to each other by its various domains^[15]. Recent research has indicated that intersectin 1 participates in both endocytosis and exocytosis and regulates different intra-cellular signaling transduction pathways. Intersectin 1 is widely reported to be involved in the process of synapse formation and endocytosis. Our previous electromicroscopy results also indicated that intersectin 1 expression was found concentrating in synapses^[5] (Fig. 3). Intersectin 1 is expressed in clathrin-coated vesicle and participates in clathrin-mediated endocytosis via its SH3 domains^[9]. It is co-localized with several proteins such as dynamin, clathrin and synaptojanin, which are well known to participate in endocytosis^[16]. One of the SH3 domains of intersectin 1 can interact with stoned-B, and this interaction

is thought to be critical for synaptic vesicle by recruiting dynamin to the pre-fission complex^[12]. During neurotransmitter release, intersectin 1 protein re-localizes from synaptic vesicle to presynaptic membrane, and then regulates dynamin release^[17]. In addition to endocytosis, intersectin 1 also functions in exocytosis. It has been reported that intersectin 1 can interact not only with endocytotic proteins, but also with SNAP-25, a protein of the exocytotic machinery that is critical for the docking of vesicles to the plasma membrane^[16]. Malacombe *et al.* provided direct evidence of a role of intersectin 1-L in exocytosis. They found that intersectin 1-L was present at the site of exocytosis of dense-core secretory granules; it also was the GEF promoting nucleotide exchange and activation of Cdc42 in response to secretagogues. Therefore, intersectin 1-L is a key component of the pathway that activates Cdc42-dependent reactions and thereby links extracellular signals to actin rearrangement required for exocytosis^[18,19].

3.2 Actin remodeling In addition to endocytosis and exocytosis, intersectin 1 is also involved in actin polymerization^[9,20]. Hussain *et al.* reported that the expression of intersectin 1 DH-domain-containing constructs caused the rearrangement of actin specific for Cdc42 activation in cultured cells. Their *in vivo* studies also revealed that stimulation of Cdc42 by intersectin 1 could accelerate actin assembly via the N-WASP and Arp2/3 complex. N-WASP binds directly to intersectin 1 and upregulates the activity of its guanine nucleotide exchange factor (GEF), thereby generating GTP-bound Cdc42, a critical activator of N-WASP. These studies revealed a role of intersectin 1 in a novel mechanism of N-WASP activation and in regulation of actin cytoskeleton.

3.3 Participation in signaling pathways A number of studies suggested that beside participation in endocytosis and exocytosis, intersectin 1 also plays an critical role in regulating intra-cellular signaling transduction pathways. Intersectin 1 can form stable protein complex with Sos through its SH3 domain, which suggests that it can regulate the level of intra-cellular Ras GTP. Meanwhile, it regulates the intra-cellular cytoskeleton structure, especially actin polymerization^[21]. Intersectin 1 has the potential to activate mitogenic signaling pathways: it activates Elk-1 transcription factor in a MEK-Erk-independent manner, which suggests that intersectin 1 can regulate cell mitosis through other MAPK signaling pathways^[22]. Several reports have indicated that intersectin 1 plays

Fig. 3 Electron micrograph of intersectin 1 positive neuron. Intersectin 1 positive vesicular structures (large arrows) and plasma membrane domains (small arrows) seemed to be distributed in dendrites (presenting as D in graph). Scale bar, 2 μ m in A and 500 nm in B. (Ma *et al.*, *J Neurosci Res*, 2003^[5])

an essential role in cell morphological regulation and maturation. Some *in vitro* studies suggest that intersectin 1 overexpression can accelerate the fibroblast formation and oocyte progesterone-induced maturation^[22]. Moreover, studies by intersectin 1 mutant constructs declared that intersectin 1 was involved in several intra-cellular signal transduction pathways, such as RTK, JNK and Ras, thereby had impact on cell proliferation and differentiation^[23]. In addition, intersectin 1 is a scaffold for the E3 ubiquitin ligase Cbl, and forms a complex with Cbl *in vivo*. This interaction stimulates the ubiquitylation and degradation of the activated EGFR^[24]. Taken together, intersectin 1 as an adaptor protein serves as a bridge between exo-endocytosis and intra-cellular signaling transduction, and it is also a key factor exerting functions in different stages of cellular processes.

4 Isoform specificity

Two isoforms of intersectin 1 probably play isoform-specific roles according to their different distributions. Northern blotting analysis reported that the ratio of mRNA of intersectin 1-L to intersectin 1-S expression in the brain increased after birth. Therefore, the expressions of intersectin 1-L and intersectin 1-S are strictly regulated in each cell or tissue type^[15].

Neurons stop mitosis after maturation in the central nervous system, so the number of neurons scarcely increases, but its special structure-synapse is increasing and plays an important role in neuronal information transfer by endocytosis and exocytosis. Glia cells as a kind of neuron nutritional supporting cells keep the division growth activity^[25,26]. We have reported that intersectin 1-L isoform is mainly expressed in neurons, and probably is involved in neuron endocytosis, exocytosis and neurotransmitter release; while intersectin 1-S isoform is mainly expressed in glia, and may participate in regulating intra-cellular signaling pathways, and therefore plays an key role in cell division and proliferation. As the expression proportion of intersectin 1-L isoform to intersectin 1-S isoform exerts an increasing tendency in postnatal, it is possible that synapse activity rises gradually after born, therefore the expressions of endocytosis and exocytosis associated proteins are gradually enhanced, and the expressions of the two isoforms during development are well consistent with their unique functions.

Until last year, few studies about isoform specific func-

tion of intersectin 1 are reported. Currently, some researchers are concentrating on establishing intersectin 1 isoform-specific gene knocking out mouse model. Yu *et al.* reported that they made the intersectin 1 total null mice, of which the short and long isoforms were lacking, and the long isoform-specific null mice, of which the long isoform was missing. They found that in both models, intersectin 1 was essential for correct vesicle trafficking. This strongly supported our hypothesis that intersectin 1-L functions in endocytosis and exocytosis. However, the distinct role of intersectin 1-S still needs further research and investigation to reveal its functions^[27].

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中枢神经系统中的桥梁分子 Intersectin 1

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摘要: 唐氏综合征(Down syndrome, DS), 也称 21 三体综合症, 由先天性染色体异常引起, 其主要特征是精神发育迟缓和先天性心脏病。所有的 DS 患者都会出现早发的阿尔茨海默病样的神经病理学特征。Intersectin 1 基因位于人类第 21 条染色体 DS 的关键区域, 且 Intersectin 1 蛋白在 DS 病人脑中有高表达, 因此全面掌握 Intersectin 1 的功能特性对进一步阐明 DS 的发病机制有重要意义。Intersectin 1 的功能不仅表现在中枢神经系统中特有的神经递质的传递, 并且还参与介导细胞增殖分化等有关的一系列信号通路的传导。本文就 Intersectin 1 在中枢神经系统中的分布、表达特征及功能作一综述。

关键词: Intersectin 1; 神经元; 胞吞作用; 胞吐作用