ARTICLE IN PRESS

Biochemical and Biophysical Research Communications xxx (2017) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Neuroligin-induced presynaptic differentiation through SLM2-mediated splicing modifications of neurexin in cerebellar cultures

Yuji Sato [a, b], Satoko Suzuki [a], Yoko Iijima [a], Takatoshi Iijima [a, *]

[a] Institute of Innovative Science and Technology, Medical Division, Tokai University, Isehara City, Kanagawa, Japan
[b] Department of Chemistry and Bioresource, Faculty of Engineering, Tokai University, Hiratsuka City, Kanagawa, Japan

A R T I C L E I N F O

Article history:
Received 11 September 2017
Accepted 18 September 2017
Available online xxx

Keywords:
Alternative splicing
GABAergic neuron
Neurexin
Neuroligin
SLM2
Synapse

A B S T R A C T

Neurexins (NRXs) and neuroligins (NLs) play important roles in synapse specification. The alternatively spliced segment 4 (AS4) of NRX genes (*Nrxn*) is a critical element in selective *trans*-synaptic interactions. However, the role of splicing of NRXs and NLs in synapse specification is not fully understood. To investigate the exact role of splice-dependent NRX–NL interaction in the specification of glutamatergic and gamma-aminobutyric acid (GABA)-ergic synapses in the cerebellum, we evaluated the synaptogenic receptor activity of NL1/2/3 isoforms in a neuron-fibroblast co-culture system, in which the *Nrxn* AS4 segments are manipulated using SLM2, a selective and dominant regulator of AS4 splicing. We show that ectopic SLM2 expression (SLM2 E/E) causes marked skipping of exon 20 of AS4 in cerebellar neuron culture. Whereas NLs can induce VAMP2$^+$ presynaptic contacts from mainly glutamatergic neurons in both uninfected (control) and SLM2 E/E co-cultures, they induce VGAT$^+$ GABAergic contacts in the control culture, but not properly in the SLM2 E/E culture. Furthermore, *Nrxn3* is responsible for the NL-induced assembly of GABAergic synapses in co-culture. Importantly, lentivirus-based expression of *Nrxn3* containing exon 20 restores the reduced NL-induced GABAergic contacts in the SLM2 E/E co-culture. Therefore, our findings may provide further insights into NRX–NL mediated synapse specification.

© 2017 Elsevier Inc. All rights reserved.

1. Introduction

Neurexins (NRX) constitute a highly polymorphic family of synaptic cell adhesion proteins, which are diversified by extensive alternative splicing. NRX variants exhibit distinct isoform-specific interactions with several *trans*-synaptic receptors. Thus, the repertoire of *trans*-synaptic NRX complexes can exert a variety of effects on the synaptic properties [1]. The incorporation of exon 20 of neurexin genes (*Nrxn*) in alternatively spliced segment 4 (AS4) generates NRX4(+) protein variants containing a 30-amino acid insertion, whereas skipping exon 20 results in the production of the NRX4(−) variant. Importantly, the alternative splicing of *Nrxn* underlies a synaptic adhesive code. More specifically, NRX4(+) and 4(−) variants interact differentially with several ligands, neuroligins (NLs), leucine-rich repeat proteins (LRRTMs), and the Cbln1-GluD2 complex [1–3]. Therefore, alternative splicing of AS4 controls a fundamental switch in the function of NRXs at synapses.

It is currently thought that this wide repertoire of NRX transcripts could contribute to shaping the morphological and functional diversity of synapses in the central nervous system (CNS). A recent study addressed the physiological significance of a single alternative splicing isoform of *Nrxn* by generating knock-in mice expressing *Nrxn3* in which the alternatively spliced exon 20 on AS4 was either constitutively included or excluded by means of genetic manipulation [4]. They demonstrated that a shift in the alternative splicing at AS4 alters excitatory synaptic transmission in the hippocampus. However, *Nrxn* is expressed from three independent genes (*Nrxn1, Nrxn2, and Nrxn3*) and two alternative promoters (α and β) [5]. Given that *Nrxn* transcripts are co-expressed in several brain areas, precisely tuned genetic manipulation of all three *Nrxn* genes within the specific type of neuron is necessary for exploring the exact role of NRX diversity in specific cell types.

The other approach would be to manipulate *Nrxn* splicing at AS4 by means of a selective splicing modifier. Notably, recent studies

Abbreviations: AS4, alternatively spliced segment 4; CNS, central nervous system; GABA, gamma-aminobutyric acid; GC, granule cell; LRRTM, leucine-rich repeat protein; ML, molecular layer; NRX, neurexin; NL, neuroligin; *Nrxn*, neurexin genes; PC, Purkinje cell; PF, parallel fiber; SPR, surface plasmon resonance; VGAT, vesicular GABA *transporter*; VAMP2, synaptobrevin.

* Corresponding author. Tokai University Institute of Innovative Science and Technology, 143 Shimokasuya, Isehara City, Kanagawa 259-1193, Japan.
E-mail address: takatoshi.iijima@tokai-u.jp (T. Iijima).

https://doi.org/10.1016/j.bbrc.2017.09.097
0006-291X/© 2017 Elsevier Inc. All rights reserved.

Please cite this article in press as: Y. Sato, et al., Neuroligin-induced presynaptic differentiation through SLM2-mediated splicing modifications of neurexin in cerebellar cultures, Biochemical and Biophysical Research Communications (2017), https://doi.org/10.1016/j.bbrc.2017.09.097

have identified a regulatory mechanism responsible for the alternative splicing of *Nrxn* AS4 in the mammalian CNS. We initially identified SAM68 and SAM-like molecule 1/2 (SLM1/2) as key players in *Nrxn* splicing at AS4 [6–8]. SAM68, SLM1, and SLM2 belong to the STAR protein family that has a KH-type RNA-binding domain [9]. While SAM68 regulates neuronal activity-dependent splicing of *Nrxn*, SLM1 and SLM2 regulate *Nrxn* splicing in a cell-type-specific manner [10]. SLM2, in particular, establishes specific regional splicing patterns of the *Nrxn* AS4 exon in the mouse brain [8,11]. SLM2 knock-out markedly impairs the skipping of exon 20 at AS4 in the forebrain, leading to the absolute lack of the *Nrxn*4(−) variant. Importantly, given that SLM2 is a unique splicing factor dedicated to specific splicing programs of a few target mRNAs that encode synaptic proteins [11,12], SLM2 is a strong, selective splicing factor for AS4, which may be useful for the modification of neural circuits through modulating the *Nrxn* splice code.

Here, we systematically evaluated the *in vitro* synaptogenic receptor activity of NL1/2/3 isoforms on HEK293 cells co-cultured with postnatal cerebellar neuron cultures ectopically expressing SLM2 (SLM2 E/E). We show that SLM2 E/E dynamically modulates NL-induced presynaptic differentiation through alternative splicing of *Nrxn* in cerebellar neurons *in vitro*. Importantly, these SLM2-mediated splicing modifications of *Nrxn* suggest the potential role of splice-dependent *trans*-synaptic signalings of NRX-NL in distinct synapse specification between glutamatergic and gamma-aminobutyric acid (GABA)-ergic cerebellar neurons *in vitro*.

2. Materials and methods

2.1. RNA isolation and alternative splicing assays

RNA was harvested from cultured neurons using Trizol reagent (Invitrogen), followed by removal of contaminating DNA using Turbo DNA-free (RNase-free DNase; Ambion, Austin, TX). One microgram of total RNA was reverse transcribed using random hexamers and ImProm-II (Promega, Madison, WI) for semi-quantitative polymerase chain reaction (PCR). The oligonucleotide primers used for semi-quantitative PCR have been described previously [7].

2.2. Lentivirus production

VSV-G pseudotyped lentiviral vectors [13] were used as described previously. The pCL20c vectors were designed to be under the control of the MSCV promoter. The viral vector was produced as described previously [6].

2.3. Neuronal cell culture

Cerebellar neuronal cultures were prepared from ICR mouse pups on postnatal day 5–7, as previously described [7]. For the knockdown experiment, the commercially available cell-permeable Accell control siRNA (individual, D-001910-01) and mouse *Nrxn3* siRNA (smart pool, E−050722-00-0005) (GE Healthcare Dharmacon, Lafayette, CO) was used. All the procedures related to the care and treatment of animals was carried out accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of Tokai University. All mice were maintained under specific pathogen-free conditions at the Laboratory Animal Center, Tokai University. The Institutional Animal Care and Use Committee of Tokai University (permit number: 174018) approved the protocol. All surgical procedures were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize the suffering of animals.

2.4. Image acquisition and analysis

Confocal images were captured on a LSM700 confocal system (Zeiss, Oberkochen, Germany). The original images were analysed using ImageJ software (NIH, Bethesda, MD). After the appropriate threshold was set, the immunoreactive areas in HEK293T cells were measured. Approximately 15–30 cells from more than 10 separate fields per culture were quantified per each group. The morphology of HEK293T cells was visualized by co-expressing green florescent protein (GFP).

2.5. Statistical analysis

Pairwise comparisons were performed using Student's *t*-test. Analysis of variance (ANOVA) was followed by Bonferroni's tests to correct for multiple comparisons. Data are represented as the mean ± standard error of the mean. Significance levels of the results are indicated as follows: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$.

3. Results

3.1. Effect of ectopic SLM2 on Nrxn splicing

Nrxn1/2/3 AS4 splicing shows a distinct pattern in various brain regions and neuronal cell types [6,10,14,15]. The splicing pattern was highly correlated with SLM2 expression [8]. Notably, mature cerebellar granule cells, which do not express SLM2, exclusively express *Nrxn*4(+) [7,16].

In order to investigate the splicing response of endogenous *Nrxn* AS4 to SLM2 in neurons that do not express SLM2, we ectopically expressed SLM2 in the cerebellar neuron culture by means of lentiviral infection at 7–8 days *in vitro* (DIV). Five days after infection, immunostaining with anti-SLM2 antibodies detected ectopically expressed SLM2 in the infected culture, while the immunoreactivity was not detected in the control culture (Fig. 1A); this confirmed that cultured cerebellar neurons infected with the lentivirus express significant amounts of SLM2 proteins. We then evaluated the shift in *Nrxn* splicing at AS4 in the SLM2-expressing cerebellar neurons by semi-quantitative reverse transcriptase (RT)-PCR, using a primer set designed to the flanking exons (exon 19 and exon 21) (Fig. 1B). We had previously demonstrated that ectopic expression of proteins from another family member, SLM1, promotes a shift in splicing to *Nrxn*4(−) in cultured cerebellar neurons [6]. The present study demonstrated that SLM2 E/E results in skipping of exon 20 of all the *Nrxn*1/2/3 even more markedly without affecting the total amount of *Nrxn* transcripts, whereas SAM68 modestly changed the splicing of *Nrxn1*, but not that of *Nrxn2* and *Nrxn3* (Fig. 1C). Additionally, SLM2 E/E did not influence splicing of any other alternative splice segments of *alpha-Nrxn1*, including AS1, AS2, AS3, AS6, and AS5 (Fig. 1D). Thus, we confirmed that SLM2 E/E selectively and absolutely alters endogenous *Nrxn* splicing at AS4, with skipping of exon 20.

3.2. Trans-synaptic interactions with post-synaptic partners

Alternative splicing of *Nrxns* at AS4 governs synaptic adhesive codes, regulating the interaction with several post-synaptic partners (Fig. 2A). While NRX4(+) forms specific *trans*-synaptic adhesion complexes with the GluD2-Cbln1 complex [16], NRX4(−) specifically forms complexes with LRRTM2 [17]. NLs can bind both the NRX isoforms, but exhibit different affinities for different isoforms [3,18]. To examine the influence of SLM2 E/E-induced switching in *Nrxns* at AS4 on *trans*-synaptic interactions with post-synaptic partners, we utilized a neuron-fibroblast co-culture system [19]. We compared *in vitro* synaptogenic receptor activity

Please cite this article in press as: Y. Sato, et al., Neuroligin-induced presynaptic differentiation through SLM2-mediated splicing modifications of neurexin in cerebellar cultures, Biochemical and Biophysical Research Communications (2017), https://doi.org/10.1016/j.bbrc.2017.09.097

Download English Version:

https://daneshyari.com/en/article/8296295

Download Persian Version:

https://daneshyari.com/article/8296295

Daneshyari.com