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Neuregulin 1-β regulates cell adhesion molecule L1 expression in the cortex and hippocampus of mice

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ABSTRACT

Neuregulin 1 (Nrg1) functions in neuronal migration, survival and differentiation as well as synaptogenesis during ontogenetic development and maintenance of synaptic functions in the adult mammalian brain. The neural adhesion molecule L1 (L1CAM) functions in similar overlapping, but also non-overlapping roles in the nervous system. In the present study, we therefore investigated some aspects of the functional relationship between Nrg1 and L1 in mammalian neural cells. Nrg1 regulates the expression of L1 in cultures of both human neuroblastoma SK-N-SH cells and mouse cortical and hippocampal neurons. To analyze the role of Nrg1 on L1 expression *in vivo*, young adult male mice received intraperitoneal injections of Nrg1 or PBS (vehicle control). The correlation between Nrg1 and L1 expression was tested by qPCR, Western blot analysis, and immunocytology. Our data indicate that neuregulin 1-β (Nrg1β) increases L1 expression in neurons of the cerebral cortex, and decreases expression in neurons of the hippocampus *in vitro* and *in vivo*. In addition, Nrg1 induces phosphorylation of its receptors, ErbB2 and ErbB4, the predominant ErbB receptors in the nervous system. These results show that Nrg1β affects expression of L1 in the central nervous system and in parallel activates the ErbB receptors for Nrg1, suggesting a crosstalk between molecules that are of prime importance for nervous system functions.

1. Introduction

Recent evidence indicates that the cell adhesion molecule L1 and the cytokine Nrg1 correlate positively in expression in gliomas, with increased L1 expression associated with increased Nrg1 expression, and with the highest expression of both molecules in glioblastomas of high grade and high tumor metastatic potential here [1]. Interestingly, the interdependence of expression of these molecules in normal nerve cells has not been studied nor is it known to which extent Nrg1 regulates L1 expression in human neuroblastoma. We therefore investigated whether Nrg1 directly influences L1 expression not only in a neuronal tumor, but also in normal neuronal cells derived from early postnatal mouse brain in dissociated primary cultures.

L1 cell adhesion molecule (L1CAM) is a 200–220 KD membrane glycoprotein first described in 1984 [2]. Accumulated evidence indicates that L1 influences the development of the nervous system. It promotes neuronal migration and survival, neurite outgrowth and synapse formation during ontogenetic development. L1 promotes migration of neurons from midbrain to the diencephalon and enhances their differentiation into the dopaminergic phenotype [3]. During cerebellar development, L1 promotes migration of granule cell neurons from the external to the internal granular layer in cooperation with NrCAM [4]. In addition, L1 can influence axon guidance through Slit and CHL1 [5]. In the adult nervous system, L1 stimulates synaptic plasticity and learning and memory [6]. Malfunction of L1 during development leads to mental retardation, aphasia, shuffling gait and adducted thumbs (MASA syndrome) and is linked to mental diseases such as schizophrenia and autism [7]. In more recent years, L1 has developed into a prominent cancer-associated adhesion molecule, with overexpression of L1 correlating positively with malignancy of glioma [8], melanoma [9], ovarian cancer [10], colon carcinoma [11] and breast cancer [12]. Thus, L1 promotes carcinogenesis [13], metastasis [14], and chemoresistance of cancer cells [15].

Interesting features in L1's functions are growth factors that regulate its expression. Among these are neuregulins which derive from alternative splicing of the neuregulin gene, which by now amount up to 15 different isoforms [16]. As a member of the epidermal growth factor (EGF)-like factor family, neuregulin 1 (Nrg1) acts as a key regulator of nervous system functions. The EGF-like domain of Nrg1 activates a series of biological processes via the specific binding with ErbB receptors [17]. Interaction of Nrg1 with ErbB receptors activates intracellular signaling pathways [18] which are involved in regulation of nervous system development and regeneration [19]. The β-type EGF-like domain of Nrg1β preferentially binds to ErbB2 and ErbB4 receptors [20],

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thereby promoting development, survival, and metabolism of neural cells [21].

In the nervous system of mammals, Nrg1 performs a multitude of functional roles in regulating many aspects of development and maintenance of normal functions in the adult [22–26]. Among the Nrg1 isoforms, the Nrg1β subtype is the most prominent one in the nervous system. It binds to ErbB2 and ErbB4 receptors the subtypes of ErbB receptors that belong to the class of receptor tyrosine kinases. Among the many types of these receptors, ErbB2 and ErbB4 are the ErbB receptors most prominently expressed in the mammalian central nervous system and together with their Nrg1 ligand contribute to neural development and functioning of the nervous system in the adult.

The functions of L1 and Nrg1 in the nervous system are strikingly similar. They promote nervous system development and functional maintenance. Outside the nervous system they contribute to tumor cell growth and spreading. Interestingly, not only Nrg1, but also L1 binds to the ErbB receptors and directly interacts with them through the Ig-like domains of L1. The L1-ErbB interaction strongly enhances the activation of these receptors and the specificity of the neuregulin/ErbB signaling pathway [27]. Furthermore, Nrg1 was recently found to enhance L1 expression [1], thus laying the basis for migration of human glioma cells. The combined findings thus suggest that the functional interrelationship between Nrg1 and L1 both *in vitro* and *in vivo* leads to multiple consequences in normal and abnormal neural cells. However, the molecular relationships between Nrg1 and L1 *in vivo* have remained unclear. We therefore investigated whether administration of Nrg1β by intraperitoneal injection into young adult mice leads to changes in L1 expression in the brain. Here we report a positive relationship between the two molecules, in that Nrg1β regulates the expression of L1 in the adult mouse brain.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (3 months old and with an average weight of 25 g) where purchased from the Guangdong Medical Laboratory Animal Center (People's Republic of China) and maintained at 25 °C under a reverse cycle of 12 h dark/light and food and water *ad libitum*. All experimental procedures were approved by the Animal Ethics Committee of Shantou University Medical College and the authorities of Guangdong province.

2.2. Nrg1β

Recombinant *Escherichia.coli*-derived Nrg1β (Thermo Scientific) was dissolved in phosphate-buffered saline, pH 7.3 (PBS) and used for cell culture and *in vivo*. For the *in vivo* experiments, Nrg1β (140 ng/kg body weight in 100 μL PBS) or the PBS vehicle control were injected intraperitoneally daily for seven days. Five mice were used as control and seven mice were used in the Nrg1β group.

2.3. Cell culture

SK-N-SH cells were purchased from the Chinese Type Culture Collection (CTCC, Shanghai, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Thermo scientific) supplemented with 10% fetal bovine serum (Sijiqing Biotech Corp) and 100 U/mL of a penicillin/streptomycin mixture (Solarbio Biotech Corp). Cells were grown in 75-cm^2 cell culture plates (Corning Inc) at 37 °C in a humidified 5% CO_2 atmosphere. To test the effects of Nrg1β on L1 expression levels *in vitro*, SK-N-SH cells (1×10^5 cells per well) in culture medium were allowed to adhere to 24-well plates (JET BIOFIL). After a 24 h incubation period, the medium was replaced with culture medium without serum, but containing Nrg1β at 0, 0.5, 1, 1.5, 2.5, 5 nM for 24 h and 48 h.

2.4. Primary culture of mouse cortex and hippocampus

Cortex and hippocampus tissues were obtained from neonatal (P0) mice as described [28]. Briefly, the hippocampus and the frontal cortex were dissected. The tissues were kept on ice in Ca^{2+}/Mg^{2+} free Hank's balanced salt solution (HBSS), followed by digestion with 0.25% trypsin at 37 °C and in a humidified 5% CO_2 atmosphere for 15 min followed by trituration and seeding into culture wells. After 6 h in culture with complete DMEM culture medium, the medium was replaced with Neurobasal-A (Life Technologies) culture medium supplemented with 2% B27 (Life Technologies), 1% L-glutamine, 1 U/mL of a penicillin/streptomycin mixture (Solarbio Biotech Corp) and 1.5 nM cytosine arabino side (Sigma/Aldrich Corp). The cells were maintained in 6-well plates (Corning Inc.) at 37 °C in a humidified 5% CO_2 atmosphere for 24 h. Then, the culture medium was replaced with Neurobasal-A medium containing 5 nM of Nrg1β and further cultured for 24 h. Cells treated with the PBS were used for control.

2.5. Tissue preparation

Mice were intraperitoneally injected with Nrg1β or PBS daily for 7 days [29]. Then, the mice were killed. Three brains, one from PBS injected mice and two from Nrg1β treated mice were fixed in 4% paraformaldehyde (PFA) and paraffin-embedded. For qPCR analysis, the cortex and hippocampus were micro-dissected, and two control and two Nrg1β-treated tissues were used to extract RNA. The residual tissues were used for Western blot analysis. The tissues were dissolved in 200 μL RIPA buffer containing PMSF (1%) (Solarbio Biotech Corp) and homogenized using a micro tissue grinder (Kimble Chase). The supernatants were collected after centrifugation at 14,000g and 4 °C for 15 min. Protein concentration was determined by the BCA assay (Solarbio Biotech Corp).

2.6. Western blot analysis

All cell and tissue lysates were combined with 20% loading buffer (LB) (0.125 mol/L Tris–HCl, pH 6.8, 20% glycerol, 10% sodium dodecyl sulfate, 0.1% bromophenol blue and 5% β-mercaptoethanol) and heated at 95 °C for 15 min. Samples were subjected to 10% SDS–PAGE and electroblotted onto polyvinylidene difluoride (PVDF) membranes (Millipore). Non-specific protein binding sites were blocked with 5% nonfat milk diluted in Tris–HCl saline buffer containing 0.1% Tween-20 (TBST, pH 7.4). Membranes were incubated with antibodies specific for mouse L1 (MAB-777, 1:1000, R&D Systems) and GADPH (1:1000, Beyotime Biotechnology) overnight at 4 °C. After 3 washings with 0.1% TBST for 5 min, horseradish peroxidase-conjugated goat anti-mouse secondary antibodies (1:1000, Boster) diluted in TBST were applied, followed by 3 washings with 0.1% TBST for 5 min each at room temperature (RT). Antigens were visualized using enhanced chemiluminescence (Beyotime Biotechnology). Signal intensity was quantified with Image Tool II software and analyzed as described [30]. Rabbit phospho-ErbB2 and phospho-ErbB4 antibodies (both at 1:500, Santa Cruz) were used to monitor changes in the activity of these receptors.

2.7. Real-time quantitative RT-PCR (qPCR)

To study L1 expression at the mRNA level in the hippocampus and cortex, total RNA was obtained from these tissues using Trizol reagent (Invitrogen) according to the manufacturer's protocol.

Then, the RNA was reverse-transcribed in a 20 μL reaction mixture with obligo-dT primers using SuperScriptTM One-step RT-PCR with Platinum-Taq DNA polymerase (Toyobo). qPCR was performed with SYBR^{R} Green Real Time PCR Master Mix (Toyobo) as described [31]. Primers for L1 [32] and GAPDH [33] were designed with Primer Premier 5.0 and prepared by Life Technologies. GAPDH was used as the loading control. The primers used for PCR are listed in Table 1.

2.8. Immunohistology

Paraffin-embedded mouse brains were cut coronally in serial sections 4 μm in thickness and were rehydrated through a graded series of ethanol to PBS. Antigen retrieval was performed using 10 mmol/L citrate buffer (pH 6.0). The samples were blocked with 10% normal donkey serum in PBS at RT for 1 h. The sections were incubated with polyclonal anti-mouse L1 antibody (1:200, R&D Systems) and rabbit anti-pErbB2 or anti-pErbB4 antibodies (both at 1:200, Santa Cruz) overnight. After 3 washings with PBS for 5 min each, the sections were incubated with donkey anti-mouse secondary antibody conjugated to Dylight^{TM} 488 (1:1000) and donkey anti-rabbit secondary conjugated antibody conjugated to Dylight^{TM} 594 (1:1000) at RT for 1 h. Confocal images for immunofluorescence were acquired using an Olympus confocal system (FV-1000, Olympus). Dylight^{TM} 488, and Dylight^{TM} 594 were excited at 488 nm, and 594 nm, respectively.

2.9. Statistical analysis

Statistical analyses were performed using the SPSS13.0 (SPSS software). Data were analyzed with the Student's *t*-test for independent samples. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Influence of Nrg1 concentrations on neuronal cells in vitro and in vivo

Nrg1β was applied to the human neuroblastoma SK-N-SH cells at concentrations between 0 and 5 nM to determine the most effective dose for L1 expression. At 24 and 48 h, a dose dependent increase in expression of L1 following Nrg1β application was seen in comparison to the PBS vehicle control, with 5 nM Nrg1β leading to the highest L1 expression (Fig. 1A and B). A dose-dependent increase in expression of p-ErbB2 and p-ErbB4 in response to Nrg1β was also observed at 24 h after exposure to Nrg1β (Fig. 1C). Compared with the PBS control, Nrg1β decreased L1 expression in hippocampal neurons, whereas a significant increase of L1 expression was observed in cortical neurons at 5 nM in 1 ml cell culture medium Neurobasal-A (Fig. 1D). Based on the results with SK-N-SH cells, 140 ng/kg per day of Nrg1β was administered to the mice daily for 7 consecutive days. Nrg1β induced a significant decrease of L1 expression in the hippocampus, while a significant increase of L1 expression was observed in the cortex (Fig. 1E).

Table 1
Primers for L1 polymerase chain reaction and product length.

Gene	Primers	Annealing temperature (°C)	Expected length (bp)
L1CAM	5′-GCCTGACACCAAATATGAGATCCACC-3′ 5′-CTGACAAAGGCGATGAACCA-3′	60	143
GAPDH	5′-GTGGAGTCATACTGGAACATGTAG-3′ 5′-AATGGTGAAGGTCGGTGTG-3′	60	150

3.2. Determination of L1 mRNA levels in hippocampal and cortical tissues after application of Nrg1β

As the Nrg1β isoform is the predominant subtype in the nervous system all experiments were performed with this molecule. L1 mRNA levels were determined by qPCR in hippocampal and cortical tissues in response to the intraperitoneal application of 5 nM Nrg1β in 100 μL PBS after seven days of daily injection. To analyze the PCR results, 1.5% agarose gel was used and results show an upregulation of L1 expression in the cortex and a decrease in the hippocampus (Fig. 2A). According to the solubility curve Ct and with $2^{-\triangle\triangle(Ct)}$, a significant increase in the mRNA level of L1 was observed compared to the PBS control with a 4.14 ± 0.51-fold increase in the cortex and a significant decrease with a 2.01 ± 0.42-fold decrease was seen in the hippocampus (Fig. 2B).

3.3. Determination of L1 expression and activated ErbB2 and ErbB4 receptors by immunohistology in cortex and hippocampus

Using immunofluorescence staining, the expression and localization of L1 in cortical and hippocampal tissues were analyzed seven days after injection of PBS and Nrg1β. In the frontal and cingulate cortices, immunofluorescence signals for L1 were increased at 5 nM compared to the PBS control (Fig. 3A). A significant decrease of L1 expression at 5 nM in comparison to the PBS control was observed in the CA3 region of the hippocampus (Fig. 3B). Moreover, levels of p-ErbB2 and p-ErbB4 were also increased in cortical tissue (Fig. 3A) while a decrease was seen in the hippocampus (Fig. 3B).

4. Discussion

In the present study, we show that the predominant nervous system isoform of neuregulins and, thus, the only one studied here, namely Nrg1β regulates expression of L1 in cultures of a human neuroblastoma and neurons of mouse cortex and hippocampus. Nrg1β enhances L1 expression in cultured neurons from forebrain and cingulate cortices, whereas it reduces L1 expression in cultured neurons of hippocampus from newborn mice. Also surprisingly and similar to the *in vitro* situation, in the adult mouse central nervous system *in vivo* Nrg1β enhances L1 expression in the frontal and cingulate cortices, whereas it decreases L1 expression in the hippocampus. The cell types that are affected by Nrg1β's ability to regulate L1 expression are neurons. The combined observations attribute to Nrg1β a novel functional feature *in vivo*, namely its ability to regulate a prominent cell adhesion molecule in neurons.

Regulation of L1 expression by the Nrg1β activated ErbB2 and ErbB4 receptors, which are the most prominent receptors of the ErbB family in the nervous system, is likely to occur by the cognate intracellular signaling cascades of the homodimers and heterodimers formed by the two receptors. Depending on the composition of the receptors, the ErbB2 monodimer activates via SHC and its downstream signaling partners GRB2, SOS, Ras, and Erk, and additionally targets molecules in the nucleus, such as Elk1 and cMyc which affect gene expression that impact on neuronal cell migration and survival. The homodimer ErbB4 affects the same downstream signaling cascade and additionally impacts on STAT5 in the cytoplasm and nucleus. A heterodimer between these two ErbB subtypes also exists in brain, but is not driven by Nrg1β, but by Nrg3, 4 which are not abundant in the nervous system [34]. L1 itself is a component in ErbB receptor signaling in that its Ig-like

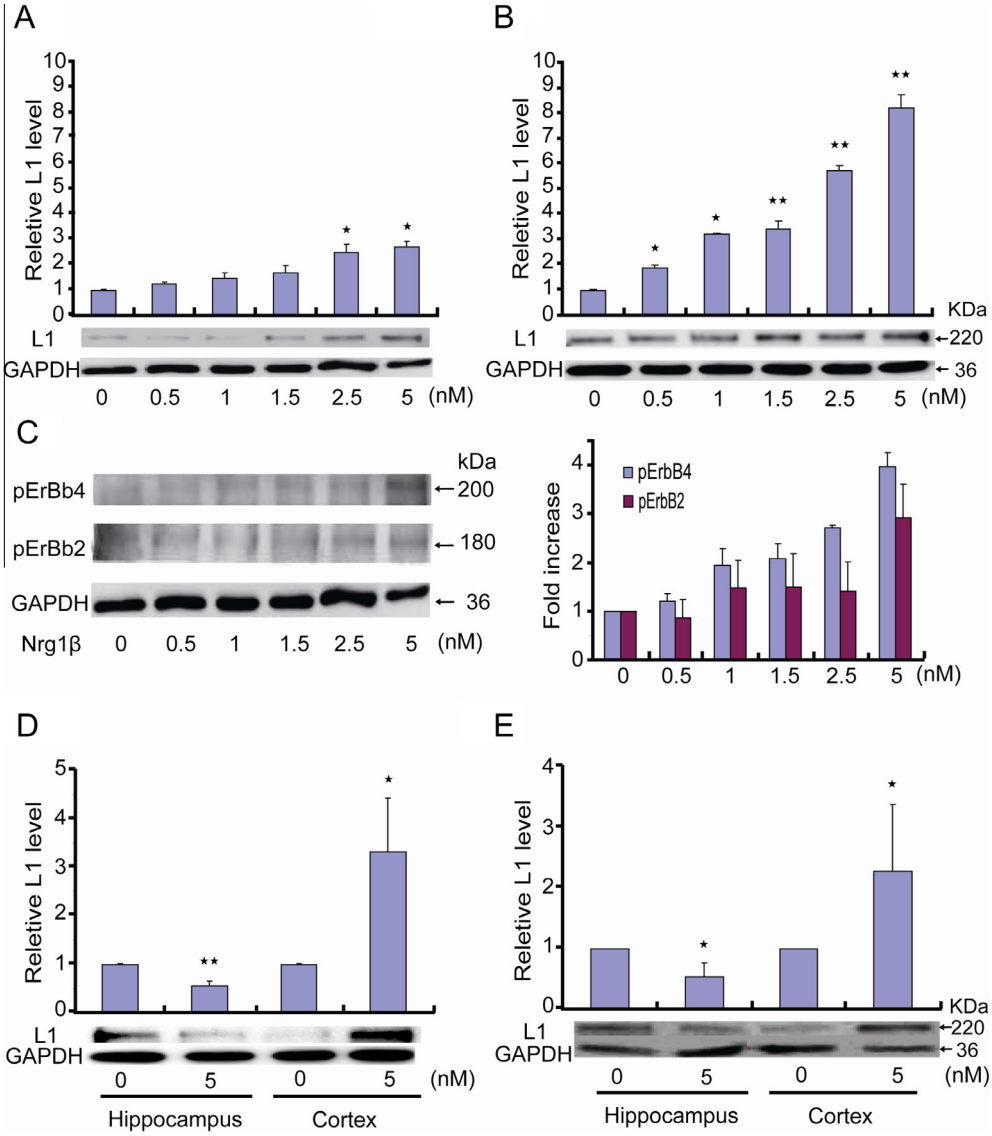

Fig. 1. Western blot analysis of L1 expression in cultured SK-N-SH cells, primary hippocampal and cortical neuronal cultures, and cortical and hippocampal tissues of adult mice. A. L1 expression in cultured SK-N-SH cells following 24 h treatment with 0–5 nM Nrg1β ($n = 3$, independent Student's t-test; mean values ± SD are shown from 3 independent experiments). B. L1 expression in cultured SK-N-SH cells following a 48 h treatment with 0–5 nM Nrg1β ($n = 3$, independent Student's t-test; mean values ± SD are shown from 3 independent experiments). C. Western blot analysis of ErbB2 and ErbB4 receptor phosphorylation level in SK-N-SH cells after 24 h of Nrg1β treatment ($n = 2$, mean values ± SD are shown from 2 independent experiments). D. L1 expression in primary hippocampal and cortical neuron cultures following treatment with 5 nM Nrg1β ($n = 3$, independent Student's t-test; mean values ± SD are shown from 3 independent experiments). E. L1 expression in hippocampal and cortical tissues in response to 5 nM Nrg1β ($n = 4$, independent Student's t-test; mean values ± SD are shown from 3 independent experiments). $^{*}P < 0.05$, $^{**}P < 0.01$ are considered as indicative of a significant difference between the Nrg1 and PBS control groups.

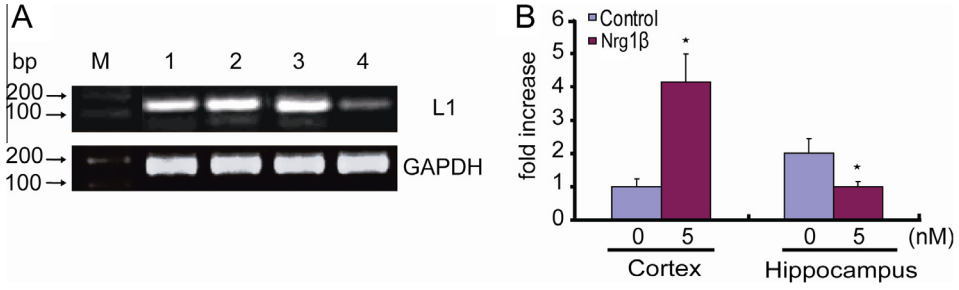

Fig. 2. L1 mRNA levels in cortical and hippocampal tissues of adult mice. A. Agarose gel electrophoresis of qPCR bands showed a significant change in L1 expression. Lane 1, control qPCR of cortical tissue; lane 2, Nrg1β-induced L1 expression in cortical tissue; lane 3, control qPCR of hippocampal tissue; lane 4, Nrg1β-induced L1 expression in hippocampal tissue. Molecular weight standards (bp) are indicated in the right (lane M). B. L1 mRNA levels, following normalization to GAPDH, in hippocampal and cortical tissues in response to 5 nM Nrg1β ($n = 3$, independent Student's t-test; mean values ± SD are shown from 3 independent experiments). $^{*}P < 0.05$ is considered as indicative of a significant difference between the Nrg1 and PBS control groups.

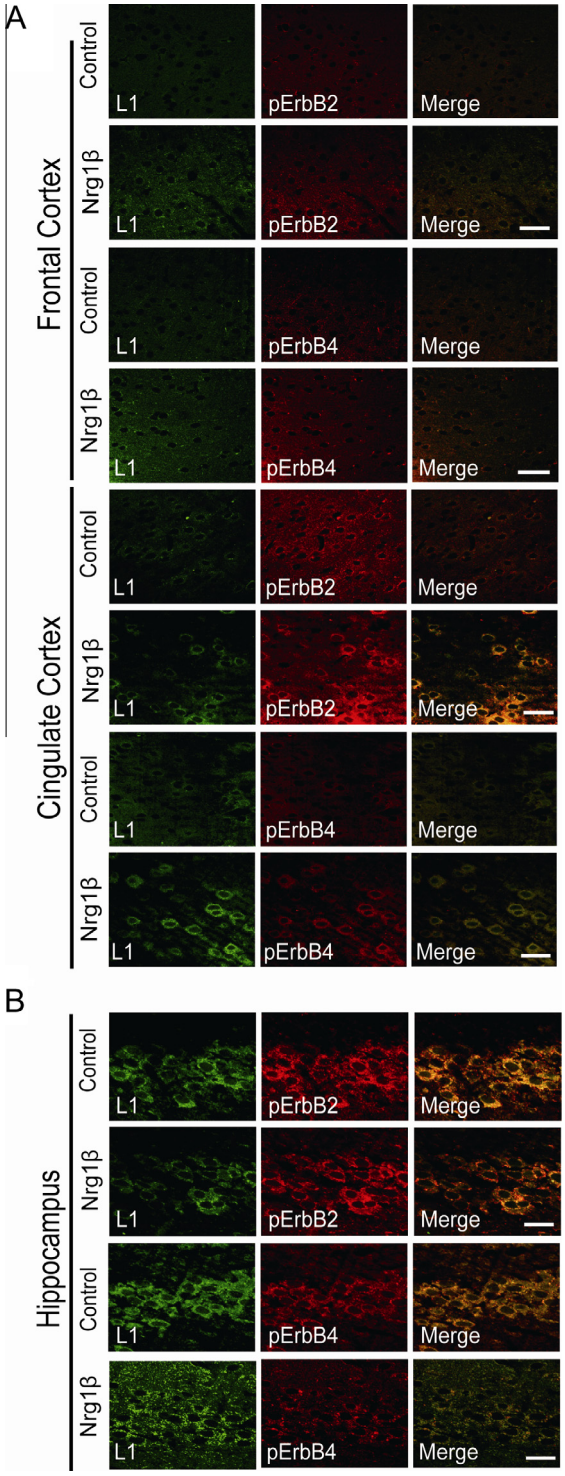

Fig. 3. Immunohistology L1, ErbB receptor phosphorylation in the cortex and hippocampus of adult mice. A. L1 expression, as well as phosphorylation of ErbB2 and ErbB4 receptors phosphorylation were increased in the frontal and cingulate cortices 7 days following intraperitoneal Nrg1β administration. B. Expression of L1, ErbB2 and ErbB4 receptor phosphorylation is reduced in the hippocampus of Nrg1β-treated mice. Immunostaining of pErbB2 and pErbB4 receptors colocalizes with L1 in the cortex and hippocampus. Scale bar = 25 μm.

domains can bind to the receptor and thus add another type of influence on neuregulin signaling. It has therefore been suggested that L1 could regulate the functions of neuregulin in a positive feedback loop [27].

Thus, the impact of Nrg1β on cortical and hippocampal neurons is likely to result from a circumscribed, yet complicated combinatorial set of receptors. The ramifications of the combined signal transduction cascades elicited by these receptors are difficult to deduce at present and will remain difficult in the near future, since knowledge on the differential expression of these receptors during distinct developmental stages in different neuronal cell types have yet to be investigated. Also, the absolute and relative concentrations of these receptors need to be known to assess the fine-tuning of signal transduction. It would be very interesting to elucidate the signaling pathways used by Nrg1β to affect expression of L1 not only during development, but also in the adult nervous system, where important readouts for L1 functions are synaptic plasticity and regeneration after trauma. The implication of neural stem cells in these important functions will be an interesting topic to investigate with the knowledge that neuregulins and L1 are implicated in shaping the cytoarchitecture and thereby the functions of the hippocampus in the adult.

It is noteworthy in these contexts that Nrg1β induces dopamine overflow in the striatum after intracerebral infusion, is neuroprotective in the hippocampus and regulates synaptic transmission in two hippocampal circuits [35]. Similarly, L1 affects survival of dopaminergic neurons in culture and in the substantia nigra in a mouse model of Parkinson's disease, is neuroprotective for all neurons investigated so far and regulates synaptic transmission and plasticity [36]. On the basis of these observations, it is not unexpected that, similar to L1, Nrg1β deficiency has been implicated in schizophrenia and mental retardation, depending on the particular site of action in the different brain regions. Interestingly, compared with the hippocampus, more ErbB receptors are localized in the cortex [37]. It is therefore conceivable that other brain regions differ considerably in the expression of ErbB receptors, thereby contributing to differential actions in brain regions that specialize for different functions. Once a precise knowledge of the expression pattern of Nrg1β and its receptors has become available, it should be possible to devise ways via which Nrg1β in conjunction with L1's functions can be incorporated into the design of therapeutic approaches in acute and chronic nervous system traumata, particularly in view of increasing incidents of neural degenerative diseases.

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References

[1] W.J. Zhao, M. Schachner, Neuregulin 1 enhances cell adhesion molecule L1 expression in human glioma cells and promotes their migration as a function of malignancy, J. Neuropathol. Exp. Neurol. 72 (2013) 244–255.

[2] F.G. Rathjen, M. Schachner, Immunocytological and biochemical characterization of a new neuronal cell surface component (L1 antigen) which is involved in cell adhesion, EMBO J. 3 (1984) 1–10.

[3] J.H. Park, G. Enikolopov, Transient elevation of adult hippocampal neurogenesis after dopamine depletion, Exp. Neurol. 222 (2010) 267–276.

[4] T. Sakurai, The role of NrCAM in neural development and disorders – beyond a simple glue in the brain, Mol. Cell. Neurosci. 49 (2012) 351–363.

[5] W. Liu, W. Zheng, B. Yuan, et al., A micropatterned coculture system for axon guidance reveals that Slit promotes axon fasciculation and regulates the expression of L1CAM, Integr. Biol. (Camb). 5 (2013) 617–623.

[6] P.F. Maness, M. Schachner, Neural recognition molecules of the immunoglobulin superfamily: signaling transducers of axon guidance and neuronal migration, Nat. Neurosci. 10 (2007) 19–26.

[7] K. Palm, N. Belluardo, M. Metsis, et al., Neuronal expression of zinc finger transcription factor REST/NRSF/XBR gene, J. Neurosci. 18 (1999) 1280–1296.

[8] W. Zhao, Comparison of L1 expression and Neuregulin-1 secretion in glioblastoma and neuroblastoma cells, Oncol. Lett. 4 (2012) 812–816.

[9] A. Thies, M. Schachner, I. Moll, et al., Overexpression of the cell adhesion molecule L1 is associated with metastasis in cutaneous malignant melanoma, Eur. J. Cancer 38 (2002) 1708–1716.
[10] S. Zecchini, M. Bianchi, N. Colombo, et al., The differential role of L1 in ovarian carcinoma and normal ovarian surface epithelium, Cancer Res. 68 (2008) 1110–1118.
[11] J.T. Kaifi, U. Reichelt, A. Quaas, et al., L1 is associated with micrometastatic spread and poor outcome in colorectal cancer, Mod. Pathol. 20 (2007) 1183–1190.
[12] T.H. Oude Munnink, W.B. Nagengast, et al., Molecular imaging of breast cancer, Breast 18 (Suppl. 3) (2009) S66–73.
[13] S. Bao, Q. Wu, Z. Li, et al., Targeting cancer stem cells through L1CAM suppresses glioma growth, Cancer Res. 68 (2008) 6043–6048.
[14] K.S. Kim, J.K. Min, Z.L. Liang, et al., Aberrant L1 cell adhesion molecule affects tumor behavior and chemosensitivity in anaplastic thyroid carcinoma, Clin. Cancer Res. 18 (2012) 3071–3078.
[15] S.S. Müerkoester, V. Werbing, B. Sipos, et al., Drug-induced expression of the cellular adhesion molecule L1CAM confers anti-apoptotic protection and chemoresistance in pancreatic ductal adenocarcinoma cells, Oncogene 26 (2007) 2759–2768.
[16] A.K. Ting, Y. Chen, L. Wen, et al., Neuregulin 1 promotes excitatory synapse development and function in GABAergic interneurons, J. Neurosci. 31 (2011) 15–25.
[17] P.J. Harrison, D.R. Weinberger, Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence, Mol. Psychiatry 10 (2005) 40–68.
[18] D.L. Falls, Neuregulins: functions, forms, and signaling strategies, Exp. Cell Res. 284 (2003) 14–30.
[19] H. Stefansson, E. Sigurdsson, V. Steinthorsdottir, et al., Neuregulin 1 and susceptibility to schizophrenia, Am. J. Hum. Genet. 71 (2002) 877–892.
[20] W.C. Dorsey, P.B. Tchounwou, B.D. Ford, Neuregulin 1-Beta cytoprotective role in AML 12 mouse hepatocytes exposed to pentachlorophenol, Int. J. Environ. Res. Public Health 3 (2006) 11–22.
[21] Z. Liu, H. Jiang, H. Li, et al., The effects of neuregulin-1β on neuronal phenotypes of primary cultured dorsal root ganglion neurons by activation of PI3K/Akt, Neurosci. Lett. 511 (2012) 52–57.
[22] N. Mechawar, B. Lacoste, W.F. Yu, et al., Developmental profile of neuregulin receptor ErbB4 in postnatal rat cerebral cortex and hippocampus, Neuroscience 148 (2007) 126–139.
[23] L. Wen, Y.S. Lu, X.H. Zhu, et al., Neuregulin 1 regulates pyramidal neuron activity via ErbB4 in parvalbumin-positive interneurons, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 1211–1216.
[24] Y. Feng, X.D. Wang, C.M. Guo, et al., Expressions of neuregulin 1beta and ErbB4 in prefrontal cortex and hippocampus of a rat schizophrenia model induced by chronic MK-801 administration, J. Biomed. Biotechnol. (2010), http://dx.doi.org/10.1155/2010/859516.
[25] A. Fisahn, J. Neddens, L. Yan, A. Buonanno, Neuregulin-1 modulates hippocampal gamma oscillations: implications for schizophrenia, Cereb. Cortex 19 (2009) 612–618.
[26] P. Fazzari, A.V. Paternain, M. Valiente, et al., Control of cortical GABA circuitry development by Nrg1 and ErbB4 signalling, Nature 464 (2010) 1376–1380.
[27] E. Donier, J.A. Gomez-Sanchez, C. Grijota-Martinez, et al., L1CAM binds ErbB receptors through Ig-like domains coupling cell adhesion and neuregulin signaling, PLoS ONE 7 (2012) e40674.
[28] S. Chen, A. Mangé, L. Dong, et al., Prion protein as trans-interacting partner for neurons is involved in neurite outgrowth and neuronal survival, Mol. Cell. Neurosci. 22 (2003) 227–233.
[29] A. Shamir, A. Buonanno, Molecular and cellular characterization of Neuregulin-1 type IV isoforms, J. Neurochem. 113 (2010) 1163–1176.
[30] W. Zhao, S.G. Ren, Neuregulin-1 (Nrg1) is mainly expressed in rat pituitary gonadotroph cells and possibly regulates prolactin (PRL) secretion in a juxtacrine manner, J. Neuroendocrinol. 23 (2011) 1252–1262.
[31] H.C. Pan, J.F. Lin, L.P. Ma, et al., Major vault protein promotes locomotor recovery and regeneration after spinal cord injury in adult zebrafish, Eur. J. Neurosci. 37 (2013) 203–211.
[32] X. Guo, L. Xiong, L. Zou, et al., L1 cell adhesion molecule overexpression in hepatocellular carcinoma associates with advanced tumor progression and poor patient survival, Diagn. Pathol. 13 (2012) 96–107.
[33] G. Falco, I. Stanghellini, M.S. Ko, Use of Chuk as an internal standard suitable for quantitative RT-PCR in mouse preimplantation embryos, Reprod. Biomed. Online 13 (2006) 394–403.
[34] L. Mei, W.C. Xiong, Neuregulin 1 in neural development, synaptic plasticity and schizophrenia, Nat. Rev. Neurosci. 9 (2008) 437–452.
[35] T. Carlsson, F.R. Schindler, M. Höllerhage, et al., Systemic administration of neuregulin-1β1 protects dopaminergic neurons in a mouse model of Parkinson's disease, J. Neurochem. 117 (2011) 1066–1074.
[36] M. Dihné, C. Bernreuther, M. Sibbe, et al., A new role for the cell adhesion molecule L1 in neural precursor cell proliferation, differentiation, and transmitter-specific subtype generation, J. Neurosci. 23 (2003) 6638–6650.
[37] D. Fleck, A.N. Garratt, C. Haass, M. Willem, BACE1 dependent neuregulin processing: review, Curr. Alzheimer Res. 9 (2012) 178–183.