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DEVELOPMENTAL REGULATION OF NEURAL CELL ADHESION MOLECULE IN HUMAN PREFRONTAL CORTEX

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Abstract—Neural cell adhesion molecule (NCAM) is a membrane-bound cell recognition molecule that exerts important functions in normal neurodevelopment including cell migration, neurite outgrowth, axon fasciculation, and synaptic plasticity. Alternative splicing of NCAM mRNA generates three main protein isoforms: NCAM-180, -140, and -120. Ectodomain shedding of NCAM isoforms can produce an extracellular 105–115 kilodalton soluble neural cell adhesion molecule fragment (NCAM-EC) and a smaller intracellular cytoplasmic fragment (NCAM-IC). NCAM also undergoes a unique post-translational modification in brain by the addition of polysialic acid (PSA)-NCAM. Interestingly, both PSA-NCAM and NCAM-EC have been implicated in the pathophysiology of schizophrenia. The developmental expression patterns of the main NCAM isoforms and PSA-NCAM have been described in rodent brain, but no studies have examined NCAM expression across human cortical development. Western blotting was used to quantify NCAM in human postmortem prefrontal cortex in 42 individuals ranging in age from mid-gestation to early adulthood. Each NCAM isoform (NCAM-180, -140, and -120), post-translational modification (PSA-NCAM) and cleavage fragment (NCAM-EC and NCAM-IC) demonstrated developmental regulation in frontal cortex. NCAM-180, -140, and -120, as well as PSA-NCAM, and NCAM-IC all showed strong developmental regulation during fetal and early postnatal ages, consistent with their identified roles in axon growth and plasticity. NCAM-EC demonstrated a more gradual increase from the early postnatal period to reach a plateau by early adolescence, potentially implicating involvement in later developmental processes. In summary, this study implicates the major NCAM isoforms, PSA-NCAM and proteolytically cleaved NCAM in pre- and postnatal development of the human prefrontal cortex. These data provide new insights on human cortical development and also provide a basis for how altered NCAM signaling during specific developmental intervals could affect synaptic connectivity and circuit formation, and thereby contribute to neurodevelopmental disorders.

Key words: NCAM, adhesion molecules, synaptic plasticity, neurite outgrowth, adolescence, schizophrenia.

Neural cell adhesion molecule (NCAM) is a membrane-bound cell recognition molecule of the immunoglobulin superfamily. NCAM contributes to normal brain development, with pleiotropic functions that include cell adhesion, cell migration, neurite outgrowth, axon fasciculation and guidance, and synaptic plasticity, as well as learning and memory (Maness and Schachner, 2007). Post-transcriptional modifications of NCAM generate three main isoforms. NCAM-180 and NCAM-140 are transmembrane forms that have long and short cytoplasmic domains, respectively. NCAM-180 is found in postsynaptic densities of mature neurons (Persohn et al., 1989; Fux et al., 2003), while NCAM-140 is expressed in growth cones and axon shafts of developing neurons (Stork et al., 2000). NCAM-120 is anchored to the cell membrane via a glycosylphosphatidylinositol (GPI) linkage and expression is primarily in glia (Noble et al., 1985). However, all NCAM isoforms may support synaptogenesis as synapses preferentially formed on cells expressing any of the three isoforms in culture (Dityatev et al., 2004). NCAM-180 and NCAM-140 are both highly expressed in the rodent neocortex during fetal and early postnatal development, and persist into adulthood at lower levels (Chuong and Edelman, 1984; Gennarini et al., 1986; Oltmann-Norden et al., 2008). NCAM-120 is found at low levels in fetal rodent brain but increases in early postnatal development and is expressed at stable levels into adulthood (Gennarini et al., 1986; Brennaman and Maness, 2008).

During embryonic development, each main isoform of NCAM can also undergo post-translational polysialylation by the addition of α-2,8-linked sialic acid residues to the fifth Ig-like domain of NCAM. Polysialic acid (PSA)-NCAM has important functions in axon growth, synaptic plasticity, and synaptogenesis (Bonfanti, 2006). Expression of PSA-NCAM is developmentally regulated in the rodent cortex,

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Abbreviations: ADAM, a disintegrin and metalloprotease; ANOVA, one-way analysis of variance; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; kDa, kilodalton; NCAM, neural cell adhesion molecule; NCAM-EC, extracellular cleaved neural cell adhesion molecule; NCAM-IC, intracellular cleaved neural cell adhesion molecule; PMI, postmortem interval; PSA, polysialic acid; TBST, Tris-buffered saline tween-20.

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with high fetal levels and rapid postnatal downregulation (Seki and Arai, 1991; Bonfanti et al., 1992; Oltmann-Norden et al., 2008; Brennaman and Maness, 2008). PSA-NCAM may also contribute to the pathophysiology of certain neurodevelopmental disorders. For example, PSA-NCAM immunoreactivity is reduced in postmortem hippocampal neurons in schizophrenia, potentially contributing to altered synaptic connectivity (Barbeau et al., 1995). Furthermore, polymorphisms in the promoter of the major sialyltransferase gene, ST8SiaII/STX, essential for NCAM polysialylation, have been associated with schizophrenia (Arai et al., 2006).

Transmembrane NCAM isoforms can undergo ectodomain shedding via a disintegrin and metalloprotease (ADAM) family of proteases, resulting in a 105–115 kilodalton (kDa) soluble extracellular domain fragment (extracellular cleaved neural cell adhesion molecule, NCAM-EC) and a smaller intracellular domain fragment (intracellular cleaved neural cell adhesion molecule, NCAM-IC) (Vawter et al., 2001; Diestel et al., 2005; Hubschmann et al., 2005; Hinkle et al., 2006; Secher, 2009). Transgenic mice that overexpress NCAM-EC in addition to full-length NCAM demonstrate marked reductions in synaptic terminals in GABAergic interneurons and pyramidal neurons and have deficits in sensory gating and amphetamine-induced locomotor activity (Pillai-Nair et al., 2005). The NCAM-EC mouse has been advanced as a potential model of GABAergic and behavioral deficits in schizophrenia, and elevated levels of NCAM-EC have been reported in the hippocampus and prefrontal cortex in schizophrenia (Vawter et al., 1998).

The pleiotropic functions of NCAM are further demonstrated by recent evidence that NCAM mimetic peptides have been found to enhance synaptic plasticity and produce neuroprotective effects in animal models (Berezin and Bock, 2009). For example, FGL—an NCAM-derived fibroblast growth factor receptor agonist—can produce reversal of working memory deficits in phencyclidine (PCP)-treated rat pups, a model of cognitive deficits with relevance to schizophrenia (Secher et al., 2009).

Although the developmental expression patterns of the major NCAM isoforms and PSA-NCAM have been described in rodent brain, we are aware of only one report in human brain in a study of PSA-NCAM in dentate gyrus (Ni Dhuill et al., 1999). The current study measured the main isoforms of NCAM (NCAM-180, -140, -120), as well as PSA-NCAM, NCAM-EC and NCAM-IC proteins in human prefrontal cortex from mid-gestation into early adulthood. Insight into the expression patterns of NCAM protein in human cortex across the first three decades of life can inform on diverse developmental processes in human brain including neuronal migration, synaptogenesis, and synaptic plasticity. Identifying the patterns of NCAM protein expression can help identify windows during which NCAM signaling may be vulnerable to developmental perturbation and provide a basis for understanding the pathophysiology of neurodevelopmental disorders such as schizophrenia.

EXPERIMENTAL PROCEDURES

Postmortem samples and tissue preparation

This study was approved by the University of North Carolina School of Medicine Institutional Review Board. Human tissue was obtained from the Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD, USA. Coronally cut slabs of prefrontal cortex (areas 9/46) from 42 human postmortem brains were frozen at autopsy and stored at −80 °C until use. Gross and microscopic examination by a neuropathologist revealed no evidence of CNS pathology. Subjects had no known history of neurological or psychiatric illness, including substance abuse. Subjects ranged in age from 18 gestational weeks to 25 years. A priori, the subjects were divided into seven age groups: fetal (n=6), 0–12 months (n=4), 1–5 years (n=6), 6–10 years (n=6), 11–15 years (n=9), 16–20 years (n=4), and 21–25 years (n=7). For subject demographics, see Table 1.

Tissue was prepared as described previously (Jarskog and Gilmore, 2000). Briefly, the tissue was homogenized (1:10, wt:vol) for 30 s on ice in homogenization buffer (50 mM Tris–HCl buffer (pH 7.4), 0.6 M NaCl, 0.2% Triton X-100, 1 mM benzamidine, 0.1 mM benzethonium chloride, and 0.1 mM PMSF) and then sonicated at 10 mV for 10 s. This method measures both soluble and membrane-bound NCAM isoforms. Samples were centrifuged for 15 min at 15,000×g and at 4 °C and supernatants were assayed for total protein using the bicinchronic acid method (Micro BCA Protein Assay Kit, Pierce Chemical, Rockford, IL, USA).

Western blotting

Samples were separated on 4% (NCAM-180, -140, -120, PSA-NCAM) or 12% (NCAM-IC) 10-well Tris–glycine polyacrylamide gels (NOVEX, San Diego, CA, USA) or 7.5% (NCAM-EC) 15-well Tris–HCl polyacrylamide gels (Bio-Rad, Hercules, CA, USA) by adapting previously established methods (Hochstrasser et al., 1988; Vawter et al., 1998; Jarskog and Gilmore, 2000). Equal amounts of total protein (10 μg for main isoforms and NCAM-EC, 100 μg for PSA-NCAM, and 55 μg for NCAM-IC) were loaded on gels with a molecular weight marker (MagicMark XP, Invitrogen, Carlsbad, CA, USA) and run at 25 °C for 90 min at 125 V, except for NCAM-EC gels that were run on ice for 35 min at 300 V. All samples were run in triplicate on separate gels. Proteins were then transferred to polyvinylidene difluoride (PVDF) membranes (Immunobilon-P, Millipore, MA, USA) at 25 V for 90 min. Complete transfers were verified by membrane staining with Ponceau S (data not shown). Nonspecific protein binding was blocked for 1 h at room temperature in 5% dry milk. Membranes were incubated with primary antibody overnight at 4 °C as follows: rabbit anti-human polyclonal NCAM (1:2000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for main isoforms and NCAM-EC, mouse anti-meningococcal monoclonal PSA-NCAM (1:200, Chemicon, Temecula, CA, USA), and mouse anti-rat monoclonal NCAM (0B11, 1:250, Sigma, St. Louis, MO, USA) for NCAM-IC in 5% milk/Tris-buffered saline tween-20 (TBST). Membranes were then incubated with secondary antibody for 90 min at 25 °C as follows: 1:3000 anti-rabbit for main isoforms and NCAM-EC, 1:1000 anti-mouse for PSA-NCAM, and 1:3000 anti-mouse for NCAM-IC in 5% milk/TBST. Membranes were developed by chemiluminescence (ECL, Amersham Pharmacia) and protein bands were detected on radiographic film (Hyperfilm ECL, Amersham Pharmacia). Images were scanned (Epson Expression, 1680) into Adobe Photoshop 7.0 (Adobe Systems, San Jose, CA, USA) and densitometry was performed using the Bioquant Nova Prime system.

Membranes were stripped using IgG Elution buffer (Fisher Scientific) and reprobed for anti–glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1:20,000, Imgenex, San Diego, CA, USA). The density of each NCAM isoform was normalized to GAPDH. Separately, increasing amounts of total protein were applied to a