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ラット水晶体組織中の神経細胞接着分子に関する免疫組織化学的研究

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Immunohistochemical Study on Neural Cell Adhesion Molecules in The Lens Tissues of Developing Rats

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Abstract

The expression of the neural cell adhesion molecule (NCAM) and its polysialic acid moiety in lens tissues was immunohistochemically examined in prenatal and postnatal rats of varying ages. In both fetuses and adults, NCAM - immunoreactivity was found in the epithelial cells of the lenses, with expression decreasing toward the bow area. These results suggest that NCAM is lost from the cell surfaces as the epithelial cells differentiate into elongating fiber cells. We have further revealed that lens epithelial NCAM is more highly polysialylated in postnatal rats than in fetal rats. In postnatal rats, highly polysialylated NCAM was localized in the lens epithelial cells around the proliferative zone, suggesting a possible role associated with cell proliferation.

Introduction

The neural cell adhesion molecule (NCAM) is a membrane glycoprotein which was primarily found in nervous tissues, and also in various types of functional cells, including glial cells, myoblasts, various endocrine cells, and cells in reproductive organs[1]. NCAM is known to have homophilic binding capabilities and influences various cell-to-cell interactions, such as axonal fasciculation, guidance of axons, cell migration, and miogenesis[2-6]. In the late embryonic and early postnatal stages, NCAM is generally present in a highly polysialylated (high PSA) form. In the course of neuronal development, the contents of sialic acid bound to NCAM peptides decreased from about 30% to less than 10% in weight, resulting in the enhanced adhesive property of the molecule[7-10].

In avian and mammalian lens tissues, NCAM localization was previously examined by Maisel and co - workers by immunohistochemical and Western blot analyses[11-13]. They revealed that NCAM was mainly expressed in lens epithelial and transition zone cells, with expression decreasing as they differentiate into the fiber cells. They also demonstrated that NCAM in avian lens epithelial cells is more highly polysialylated in adults than in the embryonic tissue[13]. The high PSA NCAM localization in lens tissues, thought, was not immunohistochemically analyzed. Therefore, in this study, we have investigated the localization of NCAM peptides and high PSA NCAM in lens tissues of rats of varying ages.

Materials and Methods

Wistar rats purchased from Charles River Japan were used for the experiments. Eyes were excised from 12-, 14-, 16- and 19- day - old fetuses, and lenses from 3-, 28- and 63-

day-old rats. The tissues were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4℃ overnight, sucrose-impregnated, and embedded in OCT compound (Miles). The middle portion of the lenses was cut at 6 μm thickness on a cryostat in the parallel planes to antero-posterial axis of the lens. The sections were mounted on egg white-coated glass slides and air-dried at room temperature.

The sections were blocked with 10% goat serum for 10min, and incubated at 4℃ overnight with a mixture of anti-rat NCAM antiserum NA1206 (AFFINITI Res. Products, 1:300) and anti-rat high PSA NCAM monoclonal antibody (MAb) 12E3 (1:5,000). NA1206 reacts with all the major forms of rat NCAM including the 180, 140 and 120K polypeptide, irrespective of PSA modification in the molecule. MAb 12E3 is mouse IgM which recognizes the PSA portion in rat high PSA NCAM, and its specificity was established previously[14,15]. After being washed with phosphate-buffered saline (PBS, pH 7.4), the sections were incubated for 30min with rhodamine-conjugated goat anti-rabbit IgG antibody (Cappel, 1:100) and fluorescein-conjugated goat anti-mouse IgM antibody (Cappel, 1:100). After the removal of unbound antibodies with PBS, the specimens were embedded in glycerol-PBS (9:1, v/v) containing 0.1% *p*-phenylenediamine (Wako), and observed under a Olympus VANOX microscope equipped with fluorescein optics. Immunohistochemical procedures were further detailed in our previous papers[16-18]. As negative controls, preimmune rabbit serum (GIBCO) and BALB/c control ascites fluid (Cedarlane Lab.) were substituted for the respective primary antibodies.

In some experiments, the sections were incubated with *Vibrio cholerae* neuraminidase (0.05 IU/ml, Sigma) in 50mM acetate buffer (pH 5.0) containing 4mM $CaCl_2$ and 0.2mM ethylenediaminetetraacetic acid at 37℃ for 2h. As a non-enzyme control, the sections were incubated with the hydrolysis solution alone. These sections were examined by double-immunofluorescence staining using MAb 12E3 and polyclonal antibody NA1206 as described above.

Results

At embryonic day 12 (E12), NCAM appeared uniformly on the surfaces of all cells in the lens vesicle (Fig. 1A). From this stage, the posterior cells of the lens vesicle begin to elongate and differentiate into fiber cells. As shown in Fig. 1C, the posterior lens cells elongated remarkably in an E14 rat lens. At this stage, immunostaining for NCAM in the lens tissue was restricted to the epithelial cells (Fig. 1C, arrows).

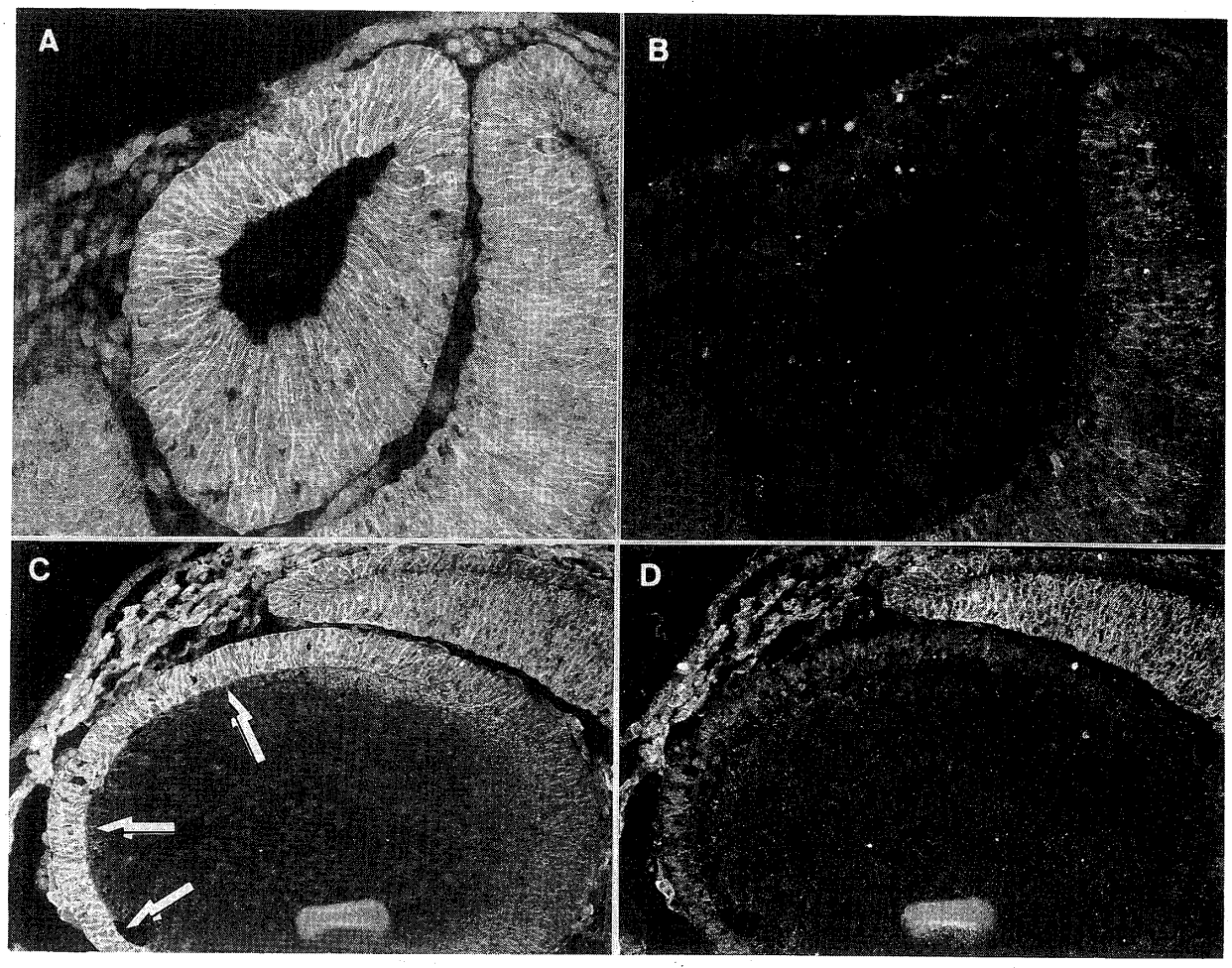

Fig. 1. Immunodetection of NCAM peptide and high PSA NCAM in fetal rat lens tissues. Sections were examined by double immunofluorescence procedures using anti-NCAM peptide (A, C) and MAb 12E3 (B, D). A,B: 12-day-old fetus. ×180. C,D: 14-day-old fetus. Epithelial cells show intense immunoreactivity for NCAM peptide (arrows in C), but not for high PSA NCAM (D). ×100.

No NCAM-immunoreactivity was detected in the cortical fiber region, suggesting that the epithelial cells lost NCAM from their surfaces during differentiation into fiber cells. This NCAM-staining pattern persisted in the lenses of later stages, including E16, E19, postnatal day 3 (P3), P28, and P63 rat lenses (Figs. 2A, 2C, 3A, 3C, and 4A) except that the immunoreaction in E19 rat lens epithelial cells was weaker than that in the other stages (Fig. 3A).

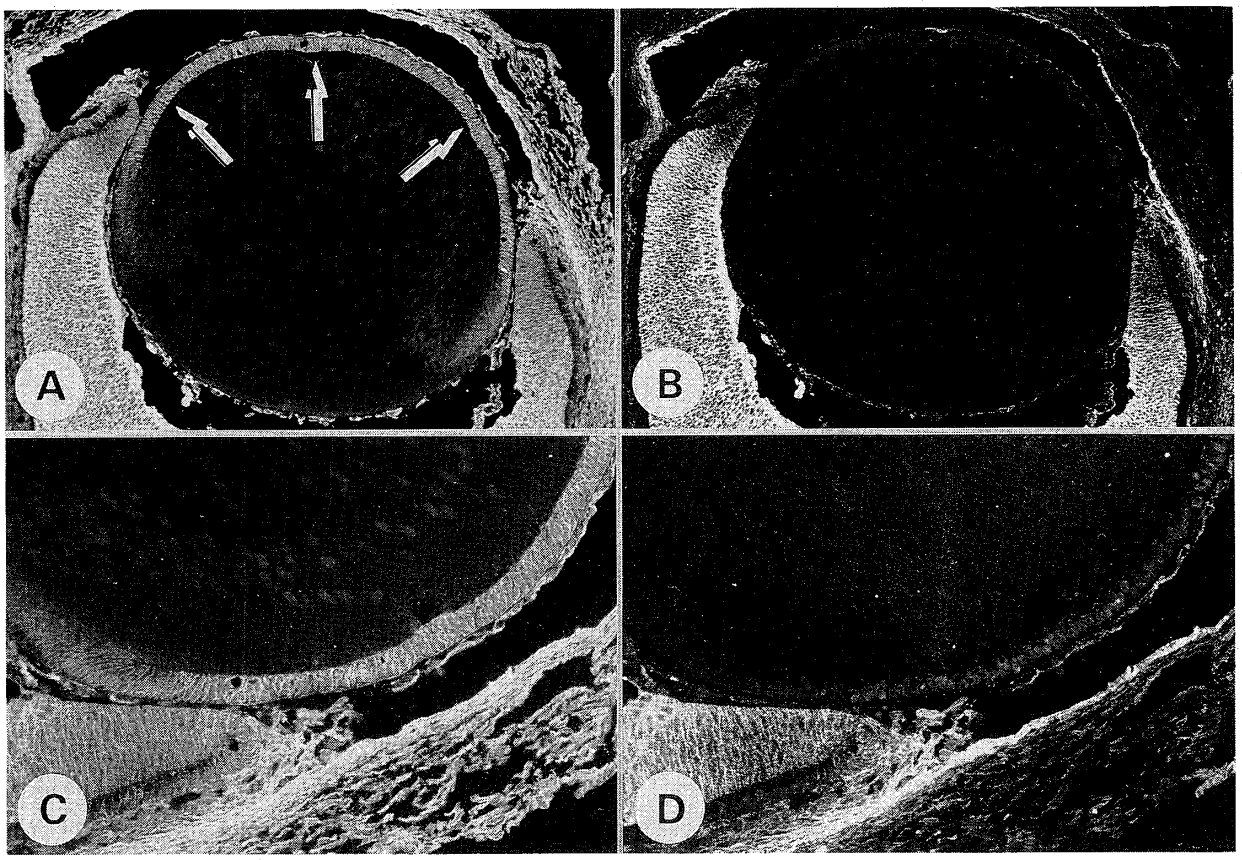

Fig. 2. Immunodetection of NCAM peptide and high PSA NCAM in 16-day-old fetal rat lens tissue. Sections were examined by double immunofluorescence procedures using anti-NCAM peptide (A, C) and MAb 12E3 (B, D). A,B: Arrows indicate the epithelial cells, which are immunoreactive for NCAM peptide. ×50. C,D: Higher magnification of A and B. ×100

To examine the localization of high PSA NCAM in the lens tissues, rat lens sections were simultaneously stained with two different anti-NCAM antibodies, NA1206 and MAb 12E3. In the lens tissues of fetal origin, no immunoreaction to high PSA NCAM was observed (Figs. 1B, 1D, 2B, 2D, and 3B). In contrast, almost all the cells in the retinas (Figs. 1D, 2B, 2D, and 3B) and optic nerve fibers (not shown) of fetal origin exhibited strong immunoreaction to high PSA NCAM.

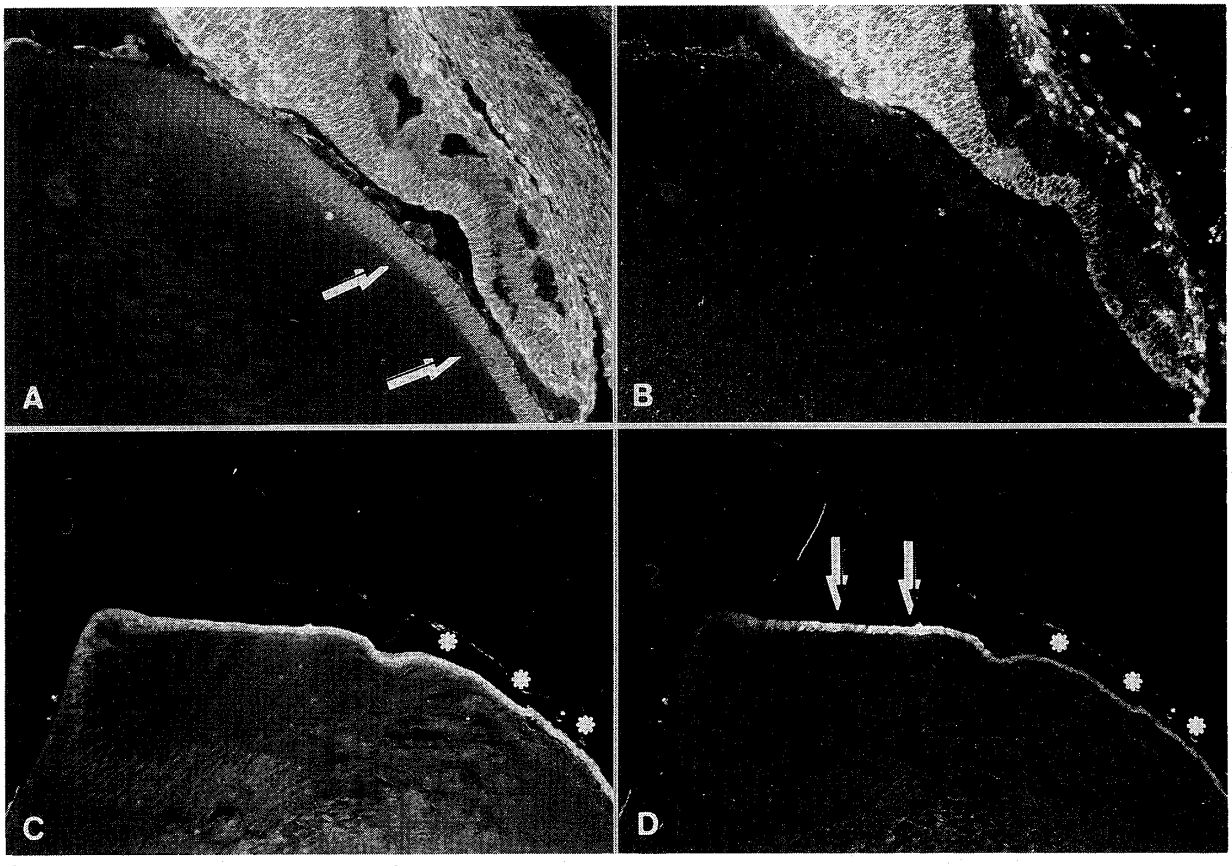

Fig. 3. Immunodetection of NCAM peptide and high PSA NCAM in fetal and neonatal rat lens tissues. Sections were examined by double immunofluorescence procedures using anti-NCAM peptide (A, C) and MAb 12E3 (B, D). A,B: 19-day-old fetus. Epithelial cells are indicated by arrows. × 100. C,D: 3-day-old rat. High PSA NCAM was localized in the epithelial cells around the proliferative zone (arrows in D). In the epithelial cells of anterior surface, immunoreactive NCAM peptide is intensely expressed, whereas no immunoreaction for high PSA NCAM was observed (asterisks). × 50.

In P3 rat lenses, a positive signal for high PSA NCAM appeared in the epithelial cells around the proliferative zone (Fig. 3D, arrows). In the epithelial cells of the anterior surface, immunoreactive NCAM peptides were intensely expressed in the cell surface, whereas no immunoreaction for high PSA NCAM was observed (Fig. 3 C and D, asterisks).

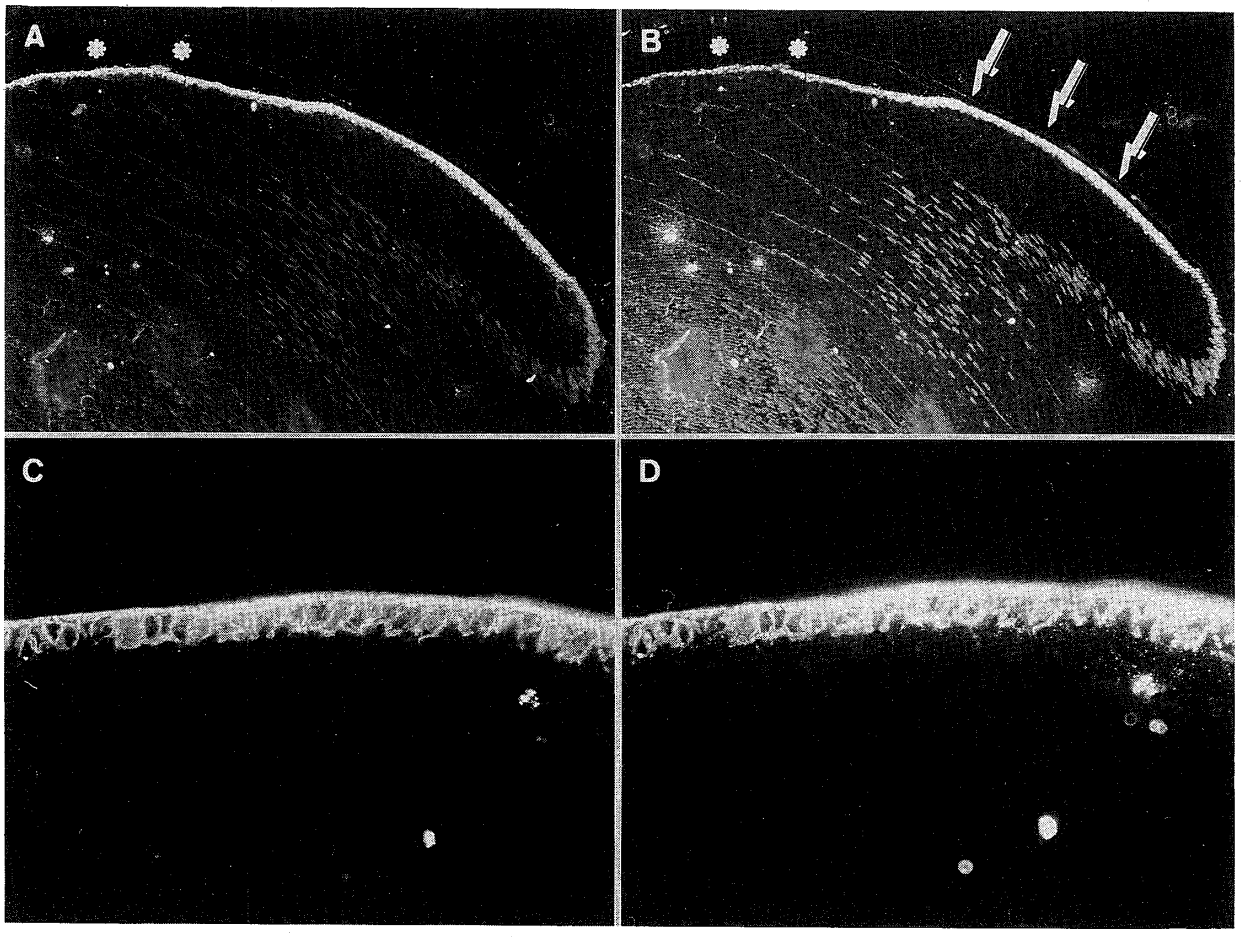

Fig. 4. Immunodetection of NCAM peptide and high PSA NCAM in 63-day-old rat lens tissue. Sections were examined by double immunofluorescence procedures using anti-NCAM peptide (A, C) and MAb 12E3 (B, D). A,B: High PSA NCAM was localized in the epithelial cells around the proliferative zone (arrows in B). In the epithelial cells of anterior surface, immunoreactive NCAM peptide is intensely expressed, whereas no immunoreaction for high PSA NCAM was observed (asterisks). At this stage, epithelial cell nuclei show strong autofluorescence. ×50. C,D: Higher magnifications of the epithelial cell sheet around the proliferative zone. Staining for NCAM peptide (C) and that for PSA (D) are strictly superimposal. ×240

Practically the same pattern of PSA localization was observed in P28 and P63 rat lenses (Fig. 4 A and B). In every section cut in the middle portion of parallel surfaces to the antero-posterial axis, the same PSA localization was observed, suggesting concentric localization along the proliferative zone. In the epithelial cells around the proliferative zone of postnatal rats, staining for NCAM peptide and that for PSA were strictly superimposal (Fig. 4 C and D), suggesting that no cells express NCAM with reduced amount of PSA, so-called "adult" forms in neurons.

Pretreatment of the lens sections with neuraminidase completely abolished immunoreactivity to MAb 12E3, whereas it did not influence the NCAM peptide immunoreactivity (data not shown). It confirmed that the immunodeterminant of the MAb 12E3 antigen is indeed the polysialic acid moiety of NCAM.

In all experiments described above, no specific immunoreaction was observed in the neg-

ative controls.

Discussion

The present immunohistochemical study revealed that NCAM is localized in the epithelial cells of rat lens tissues. These results are consistent with a previous report by Katar *et al.*, who analyzed NCAM in adult rat lenses by Western blotting[11]. In chick lenses, a similar distribution of NCAM was previously reported by Watanabe *et al*[12]. These results suggest that NCAM in general localizes in the epithelia of lenses. It is well-known that NCAM participates in cell-to-cell adhesion via the homophilic binding capability of its peptide moiety. Therefore it is likely that NCAM in lens epithelia is involved in the organization of the epithelial cell sheet. As Watanabe *et al*. previously suggested, NCAM in the epithelial cells may also facilitate the formation of gap junctions and adherence junctions by bringing cell membranes in close apposition[13]. If this is the case, NCAM is no longer required after the epithelial cells differentiate into fiber cells, which have mature gap and adherence junctions. Actually the expression of NCAM peptide is restricted to the epithelia and disappears toward the elongating fiber region.

In E19 fetuses, NCAM expression in lens epithelial cells was found to be less intense than that in E16 and P3 lenses. At present, we do not have a plausible explanation for its transient suppression of NCAM expression. Precise study on the changes in NCAM expression during these stages is now under way.

In several tissues, such as those in the peripheral and central nervous systems, skeletal muscle and thyroid gland, NCAM in the fetuses is more highly polysialylated than that in adults[5,8,9]. Therefore high PSA NCAM is sometimes called "embryonic" NCAM. Contrarily, in chicken lenses, "embryonic" NCAM is not expressed in the embryos but is present in adults[13]. The present results confirmed that this paradoxical phenomenon is also the case in rat lens tissues. Furthermore, I have found the distribution of high PSA NCAM in the epithelial cells around the proliferative zone in postnatal rat lenses, with expression decreasing toward both the central region and the bow area. Although the precise role of PSA in NCAM is not fully understood even in the nervous system, it is deduced that PSA participates in cell surface movement and cell recognition[19]. In adult brains, high PSA NCAM is not expressed in most neurons, but is expressed in the olfactory bulb and hippocampal dentate gyras[20], where neurogenesis continues throughout the adult stage. In these regions, high PSA NCAM localization is restricted to newly generated cells during the adult stage, and is lost in the early period of the developmental process[20]. In rat lens epithelium, high PSA NCAM was localized in proliferative zone where cell proliferation continues throughout postnatal ages. Therefore it is deduced that high PSA NCAM expression is associated with cell proliferation and consequent cell migration. Further experiments are required for understanding the roles of NCAM and its PSA portion in the lenses.

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