Expression of NCAM recapitulates tubulogenic development in kidneys recovering from acute ischemia

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Abbate, Mauro, Dennis Brown, and Joseph V. Bonventre. Expression of NCAM recapitulates tubulogenic development in kidneys recovering from acute ischemia. Am. J. Physiol. 277 (Renal Physiol. 46): F454–F463, 1999.—Recovery of the kidney from acute renal failure relies on a sequence of events including epithelial cell dedifferentiation and proliferation followed by differentiation and restoration of the functional integrity of the nephron. The factors responsible for, and the significance of, reversion to a less differentiated cell phenotype and its relationship to the proliferative response after ischemia are poorly understood. In an attempt to identify adhesion molecules that may be influential in the recovery process, the expression of neural cell adhesion molecule (NCAM) and markers of epithelial differentiation and proliferation were analyzed at various times after an ischemic insult. In maturing nephrons, NCAM is detectable by immunohistochemistry in renal vesicles, S-shaped bodies, and early tubules. There is minimal cellular NCAM expression in normal tubules of the adult kidney. In contrast, in postischemic kidneys, NCAM expression is abundant in S3 proximal tubule cells 5 days after reperfusion. As in developing tubules, NCAM is concentrated in basal and lateral aspects of cells that have no apical gp330 or dipeptidyl peptidase IV detectable on their brush border. The expression of NCAM is preceded by disassembly of the brush border and proliferation of surviving S3 cells, which is most prominent at 2 days postischemia. NCAM expression persists in some flattened and dedifferentiated cells for up to 7 wk after ischemia. Thus proximal tubule epithelial cells of the postischemic kidney express NCAM in a pattern that recapitulates the expression of NCAM in the developing kidney. Such reversion of phenotype extends at least back to the early stages of renal vesicle formation, and this reversion may represent a critical step in the reestablishment of a normal tubule. NCAM-matrix interactions may mediate the motogenic and mitogenic responses of the dediffereniated epithelium that are critical to reestablishment of a functional proximal tubule.

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philic cell-cell interactions (2, 11). It is expressed transiently by cells destined to form the nephron and is implicated in morphogenetic processes. In embryonic kidney, NCAM is present in cells of the metanephric mesenchyme (16, 24, 27) that are induced to aggregate and to increase in density by the ureteric bud, an outgrowth of the Wolffian duct. The cells subsequently proliferate and differentiate by a process of epithelial conversion to form the glomerulus and the proximal and distal tubular segments (16). During conversion to more mature phenotypes, NCAM is rapidly downregulated (26, 27, 38), and the mesenchymal origin of the tubular cells can no longer be recognized. Furthermore, NCAM is expressed in dedifferentiated cells of Wilms' tumors, an embryonic type tumor of the kidney (37). These patterns of expression define NCAM as a dedifferentiation marker for renal cells, and it has been used as such in studies of models of metanephric mesenchyme (16, 38) and in cultured metanephric cells (24).

The experiments described in the present report were designed to establish the following: 1) whether postischemic proximal tubular epithelial cells express NCAM, and to compare this expression to that observed in the primitive nephron; and 2) how the phenotypic reversion relates temporally with mitotic activity in the proximal tubule. Given the potential roles of NCAM in controlling cell shape and migration (42) and epithelial polarity (16), NCAM expression was analyzed in parallel with proximal tubule apical membrane proteins that are reassembled on the brush border in the postmitotic phase after ischemia.

**METHODS**

**Chemicals**

Paraformaldehyde was purchased from Electron Microscopy Sciences (Fort Washington, PA); BSA, L-lysine HCl, n-propyl gallate, 5-bromo-2'-deoxyuridine (BrdU), fluorodeoxyuridine (FDU), and DNase I (EC 3.1.21.1) were from Sigma (St. Louis, MO). Sodium metaperiodate was from Fisher Scientific (Fair Lawn, NJ), FITC-conjugated wheat germ agglutinin (WGA) lectin was from Vector (Burlingame, CA), and OCT embedding medium was from Miles (Naperville, IL).

**Animals**

Sprague-Dawley male rats (190–260 g; Charles River Breeding Laboratories, Wilmington, MA) were housed under alternating 12-h cycles of light and dark and were allowed free access to food and water. One-day-old rats of the same strain were also studied. The animals were maintained and experiments conducted in accord with the National Institutes of Health (NIH) “Guide for the Care and Use of Laboratory Animals” (DHHS Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892).

**Induction of Renal Ischemia and Reperfusion Injury**

Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium solution (6.5 mg/100 g body wt) and then injected intraperitoneally with 10 ml of 0.9% saline warmed to 37°C. The animals were placed on a heated operating board, and the left renal artery and vein were clamped with a microaneurysm clamp (Roboz Surgical Instrument, Washington, DC) via a left flank incision. Ischemia was maintained for a time period of 40 min, and the incision was temporarily closed until removal of the clamp at the end of the period of vascular occlusion, then sutured. Animals with reperfusion periods of at least 24 h were allowed free access to food and water until death. After the stated time of reperfusion, the rats were anesthetized, and kidneys were perfused via the abdominal aorta for fixation (as described below, see Preparation of Tissue for Immunohistochemistry). Three rats per group were analyzed for each time point after ischemia.

**In Vivo BrdU Incorporation**

Animals were administered BrdU intraperitoneally at a concentration of 10 mg/ml in 0.9% saline (5 mg BrdU per 100 g body wt). BrdU is an uridine analog incorporated into DNA in place of thymidine during the S-phase of cell cycle. To achieve optimal BrdU labeling, the BrdU solution was supplemented with 1 mg/ml FDU, which inhibits endogenous thymidine incorporation. In preliminary experiments, we established that the relative proportion of BrdU-positive nuclei was maximal in postischemic kidneys at 2 days postischemia, in agreement with our previous description of proliferating cell nuclear antigen (PCNA) expression in this model (45). We focused on 2-day postischemic kidneys, to examine the renal distribution of nuclear BrdU labeling and to analyze the expression of proximal tubule apical marker proteins in the cells positive for nuclear BrdU. A first group of rats received a single injection of BrdU, 4 h before the end of the 48-h reperfusion period. In another set of experiments, designed to label a greater proportion of cells, the animals were given two BrdU injections, 8 and 2 h before fixation of the kidneys at 48 h after reperfusion.

**Preparation of Tissue for Immunohistochemistry**

Kidneys were perfused via the abdominal aorta with Hanks' balanced salt solution at 37°C and then with paraformaldehyde-lysine-periodeate (PLP) (29) for 10 min. Tissue samples were further fixed by immersion overnight at 4°C in PLP. Kidneys from 1-day-old rats were fixed in PLP by immersion overnight at 4°C. The fixed tissue fragments were rinsed with PBS (0.9% NaCl in 10 mM sodium phosphate buffer, pH 7.4), and stored at 4°C in PBS containing 0.02% sodium azide until further processed. For sectioning, specimens were rinsed again in PBS, infiltrated with 30% sucrose and sodium azide until further processed. For sectioning, specimens were cooled to 20°C or stained immediately.

**Antibodies**

Mouse anti-NCAM monoclonal antibody (5B8) (obtained from Developmental Studies Hybridoma Bank, University of Iowa) was made against rat embryonic spinal cord membranes. 5B8 antibody is an IgG1 (k light chain) isotype antibody (12) that reacts with NCAM bands at 140 and 180 kDa and with the 200-kDa sialylated form on Western blots of neural tissue. 5B8 has been used for immunohistochemical studies on cultured cells, including NRK cells from rat kidney, showing identical results to other purified monoclonal and polyclonal anti-NCAM antibodies (5). The production and specificity of rabbit anti-gp330 antisemur have been described (19). Anti-rat dipetidyl peptidase IV (DPP-IV) is a rabbit polyclonal antisemur raised against rat liver DPP-IV (22) and was a gift of Dr. Ann Hubbard (Johns Hopkins University).
University School of Medicine, Baltimore, MD). It recognizes a band at 100 to 110 kDa on immunoblots of rat renal brush-border membrane vesicles (1). Rabbit polyclonal anti-heparan sulfate proteoglycan (HSPG) antiserum, a kind gift of Dr. Jenny Stow (University of Queensland, Brisbane, Australia), reacts with the core protein of rat HSPG and strongly delineates the tubular basement membranes by indirect immunofluorescence staining of sections of PLP-fixed kidneys. Tetramethylrhodamine isothiocyanate (TRITC)-conjugated anti-mouse IgG, Cy3-conjugated donkey anti-mouse-IgG, and FITC-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) were used as secondary antibodies.

Immunohistochemistry

The sections were bathed in PBS and blocked with 1% BSA/PBS for 20 min. Primary antibodies were used at the following dilutions in PBS: monoclonal 5B8 supernatant, 1:2 (−35 μg/ml); anti-gp330 monoclonal antibody (asctic fluid, 1:250; anti-DPP-IV or anti-gp330 rabbit polyclonal antibodies, 1:100; and anti-HSPG, 1:50. After overnight incubation at 4°C with primary antibody, the sections were washed twice for 5 min each in PBS (8). Sections were then treated with 0.1% SDS in PBS for 20 min to expose antigenic sites, and washed for three periods of 5 min each in PBS (8). Sections were then incubated with TRITC-conjugated goat anti-mouse IgG, rabbit immune serum (anti-DPP-IV, -gp330, or -HSPG), and FITC-conjugated goat anti-rabbit IgG. Each antibody was applied to sections under the same conditions as previously described. Other sections were incubated with anti-NCAM antibodies followed by 20 min at room temperature with FITC-conjugated WGA lectin (diluted 1:400 in PBS). Washes after each round of staining were again in HS-PBS and in PBS. In control experiments, the incubation sequences were inverted, or primary antibodies were omitted and substituted with nonimmune serum. Photographs were taken on Kodak T-Max 400 film push-processed to 1600 ASA (Eastman Kodak, Rochester, NY).

Detection of BrdU in Kidney

Five-micrometer-thick frozen sections of PLP-fixed tissues were stained with mouse monoclonal anti-BrdU antibody (Amersham, Arlington Heights, IL) according to a protocol recommended by the manufacturer, with modifications. Using this protocol, identical results were obtained with other anti-BrdU antibodies purchased from Becton-Dickinson (San Jose, CA) and Zymed (San Francisco, CA). Sections were first immersed 5 min in PBS, then in a 1% SDS solution in PBS for 20 min to expose antigenic sites, and washed for three periods of 5 min each in PBS (8). Sections were then treated with 0.1% Triton X-100/PBS for 5 min, and blocked in 1% BSA/PBS for 10 min. Anti-BrdU antibody (diluted 1:4 in 0.25 M Tris buffer, pH 7.5, containing 1 M NaCl, 50 mM MgCl2, 0.1% Triton X-100, and 0.2 mg/ml DNase) was applied to sections for 2 h at room temperature, followed by washes in HS-PBS and PBS. Sections were then incubated with TRITC-conjugated goat anti-mouse IgG for 1 h at room temperature, washed, mounted, and examined as described above. Double staining for BrdU and apical marker proteins was performed by a sequence of the individual protocols, using TRITC-conjugated goat anti-mouse IgG and FITC-conjugated goat anti-rabbit IgG as secondary antibodies.

RESULTS

Developmental expression of NCAM: Distribution of NCAM in Normal Rat Kidney

Kidneys taken from 1-day-old rats were analyzed to examine the patterns of NCAM expression in the developing nephron. The cells of the renal vesicles and S-shaped bodies stained heavily with the 5B8 monoclonal NCAM antibody in a linear pattern at the periphery of the cell (Fig. 1A). In the S-shaped body, the staining was present in cells destined to be visceral and parietal epithelial cells in the mature glomerulus, as well as cells of early tubular stages, including the proximal portion of the developing nephron (Fig. 1A and B). As shown in Fig. 1B, NCAM labeling was concentrated at the basal and lateral cell borders, reflecting plasma membrane association. Smaller amounts of immunoreactive NCAM were detectable in portions of the branching ureteric bud epithelium (not shown). In subsequent stages of nephron development, NCAM staining persisted in glomerular parietal epithelial cells; however, it was undetectable in many tubules at more advanced stages of maturation (Fig. 1C). The 5B8 antibody also stained regions of the interstitium of the medulla, particularly in the papilla, whereas epithelial cells did not stain (Fig. 1D). The staining patterns with this antibody, which recognizes both the major 140- and 180-kDa isoforms and the 200-kDa sialylated form of NCAM, are consistent with tubular cell NCAM expression restricted to primitive phases of development, with rapid disappearance of tubular cell NCAM after the S-shaped body stage.

In normal kidneys of adult rats, there was no NCAM staining in the cortex, with the exception of glomerular parietal cells that were stained predominantly at the basal and/or lateral aspects of the cells (Fig. 2A). In the medulla tubular epithelial cells did not stain for NCAM (Fig. 2B), but interstitial cells displayed NCAM staining both in the outer stripe of the outer medulla (Fig. 2B) and in deeper medullary regions (not shown).

Expression of NCAM in Kidney After Ischemia and Reperfusion

Injury and regenerative phase. Both early after reflow (1 and 3 h) and during the initial proliferative phase (24 h posts ischemia) NCAM was either undetectable in tubular cells or was seen in small amounts in the cytoplasm of some proximal tubule epithelial cells. NCAM staining at these time points was indistinguishable in postischemic, normal, and contralateral kidneys. In kidneys examined 48 and 72 h after ischemia, no NCAM was present in the severely injured S3 cells of the outer stripe nor in most other tubular epithelial cells. However, staining was sometimes found along the lateral aspect of scattered individual cells of proximal tubules in the cortex (Fig. 3A). Double immunofluorescence labeling for NCAM and either an apical mem-
brane protein of proximal tubule or WGA lectin-binding sites (Fig. 3B) revealed linear interruptions of the apical staining at the luminal border of these scattered NCAM-positive cells. NCAM was never detectable in sloughed cells or in luminal debris at this as well as any other time period of reperfusion. The interstitium exhibited a generalized increase in the intensity of staining both in the outer stripe of outer medulla (Fig. 3C) and in the deeper medullary regions. In the inner medulla, stained cells were identified as interstitial

Fig. 1. Immunofluorescence micrographs of 5-µm cryostat sections of paraformaldehyde-lysine-periodate (PLP)-fixed kidney of 1-day-old neonatal rat. Sections were incubated with 5B8 anti-NCAM antibodies with tetramethylrhodamine isothiocyanate (TRITC)-conjugated anti-mouse IgG used as a secondary antibody. A: renal vesicles immediately beneath the capsule and comma-shaped and S-shaped bodies are strongly labeled. Pattern is linear both in the progenitor cells of the tubular epithelium and those of the visceral or parietal epithelium of the glomerulus. B: in structures sectioned longitudinally, including a proximal portion of developing tubule (arrow), NCAM staining is concentrated in the basal and lateral cell borders. C: many tubules in a deeper cortical area display no NCAM immunoreactivity, but a few less mature tubules are labeled. D: papilla shows strong NCAM staining of interstitial cells. Tubules are unstained. In this and all subsequent Figs. 2–7, the results presented are representative of the findings in at least 3 animals studied in each group. NCAM, neural cell adhesion molecule. Bars = 15 µm.

Fig. 2. Cryostat sections of kidney from normal adult rat, stained by indirect immunofluorescence for the presence of NCAM (5B8 monoclonal antibodies, TRITC-conjugated anti-mouse IgG). A: a cortical area reveals staining of some glomerular parietal cells. Proximal tubules (PT) are not stained by anti-NCAM antibodies. B: some interstitial cells stain in the outer stripe of the outer medulla. There is no NCAM staining detectable in tubular epithelial cells. G, glomerulus. Bars = 15 µm.
cells arranged in rows between adjacent tubules and vessels (Fig. 3D).

Differentiation phase. At 5 days of reflow, there was marked staining for NCAM in tubules in the outer stripe of the outer medulla and in medullary rays. The staining appeared to be restricted to proximal tubules and revealed irregular patterns of cellular distribution. Most epithelial cells stained for NCAM in tubules in the deep outer stripe (Fig. 4A), the site of most severe damage and regeneration in this model of reperfusion injury (45). Other tubular profiles both in the outer stripe and in cortical medullary rays were lined by groups of cells that stained brightly for NCAM and were adjacent to NCAM-negative cells (Fig. 5A). As in the developing tubular epithelium, most of this staining was basolateral or was concentrated in either the lateral or basal borders of the cells. In some cells the staining occurred in a vesicular intracellular pattern. Exceptionally, NCAM was detectable at apical sites, but only in areas of contact with sloughed cells or luminal debris.

Relationship of NCAM Expression to Presence of Apical Proteins on Brush Border of Proximal Tubules

The reorganization of the apical region of the cell is a key process in the structural repair of the proximal tubule after ischemia. To examine the relationship between the expression of NCAM and the distribution of apical marker molecules on the brush-border membrane, sections of 5-day postischemic kidneys were double-stained for the presence of NCAM and either an apical brush border protein of proximal tubule (gp330, DPP-IV) or WGA lectin-binding sites. Mutually exclusive patterns of staining between NCAM and each of these markers are found. The complementary sets (A and B) in Figs. 3–5 clearly document that the cells expressing basolateral NCAM at 5 days postischemia did not stain at all, or only weakly, for these proteins at the apical aspect of the cells. This was evident in the majority of S3 segment cells that stained for NCAM deep in the outer stripe; Fig. 4, A and B, shows the weak staining for gp330 in the NCAM-positive cells of tu-
Fig. 4. Immunofluorescence staining of NCAM in a cryostat section of outer medulla of postischemic kidney, fixed at 5 days after reperfusion. In an area of the outer stripe, cells of many tubular profiles are NCAM positive mainly in linear patterns (A). Morphology and relative proportion of tubules in postischemic outer stripe identified these as S3 segments of proximal tubules. By double staining of the section with anti-gp330 antibodies (B), NCAM-positive tubule cells express little or no apical gp330. In some tubules, casts are intensely stained for gp330. Bars = 15 µm.

bules. Conversely, cells expressing apical membrane glycoproteins (Fig. 3B, WGA-lectin-binding sites; Fig. 5B, DPP-IV) displayed no basolateral NCAM staining. NCAM was no longer detectable in most tubular epithelial cells in kidneys taken at later time periods after ischemia. In tubules at 16 days after reflow, the only staining for this protein was in flattened cells lining the walls of a few tubules (Fig. 6A). These NCAM-positive cells were devoid of apical gp330 and DPP-IV (not shown). Tubules containing flattened cells were also found in kidneys at 7 wk after ischemia. NCAM staining was irregularly distributed either in a linear pattern or in granular structures in the cytoplasm of flattened cells (Fig. 6B). Proximal tubule marker proteins were detectable, albeit weakly, at the apical pole in some cells in the same tubules (Fig. 6, B and C).

Reexpression of NCAM in Proximal Tubule After Ischemia Follows Diffuse Proliferation of the S3 Proximal Tubular Cells That Survive Injury and Dedifferentiate

The mitogenic response of the kidney to reperfusion injury is maximal in S3 segments of proximal tubule at 48 h and almost completed at 5 days after a 40-min ischemic period (45). The similar distribution of NCAM-positive cells at 5 days and of proliferating cells at 2 days after ischemia suggests that the S3 cells expressing NCAM had divided during the regenerative phase. In addition, a great proportion of mitotically active S3 cells at 2 days stain for vimentin, consistent with cell dedifferentiation coincident with proliferation (45). To further characterize morphological events of dedifferentiation in regenerating epithelium, and to establish whether a pool of cells may exist that are induced to

Fig. 5. Immunofluorescence micrographs of an area of the medullary ray in cortex of a 5-day postischemic kidney. Tissue section was stained both with anti-NCAM (A) and anti-DPP-IV antibodies (B). In A, there is heterogeneous patterns of NCAM staining in proximal tubular cells; some of the cells stain strongly in linear and basolateral patterns; others either contain granules of staining or do not stain. Cells consistently reveal mutually exclusive patterns of basolateral NCAM in A and apical DPP-IV staining in B. DPP-IV, dipeptidyl peptidase IV. Bars = 15 µm.
divide and lose at the same time their highly differentiated apical domain, we analyzed, by double staining, the patterns of nuclear BrdU incorporation in parallel with the staining patterns of apical proteins in proximal tubules in the 2-day postischemic kidney.

When animals were given BrdU twice, at both 8 and 2 h before death, most of the cells in S3 segments had BrdU-fluorescent nuclei or mitotic figures. Figure 7 demonstrates, in a section double-stained for BrdU (Fig. 7A) and gp330 (Fig. 7B), the intense staining of nuclei of S3 cells in damaged S3 segments and the sparse BrdU labeling of nuclei in gp330-positive proximal tubules in which the brush border is retained. Very few nuclei were BrdU positive in the sections of control contralateral kidneys (not shown). In contrast to the regenerating epithelium, no BrdU was seen in sloughed cells and debris contained in the tubule lumen. In contrast, cell debris in the tubule lumen stained positively for gp330.

**DISCUSSION**

In the recovery phase after ischemic injury, the kidney moves sequentially through stages of proliferation, dedifferentiation, and differentiation. Our observations demonstrate the expression of NCAM in tubular cells during early stages of nephron maturation (16, 27, 31, 38) and after renal ischemia. Thus a molecule not expressed by normal mature renal tubule epithelial cells is expressed in proximal tubular cells during recovery from ischemia, recapitulating its expression in early renal development. Postischemic expression of NCAM is temporally associated with the reestablishment of the normal architecture of the tubule in the postmitotic phase of repair and is consistent with a functional role for this adhesion molecule in the epithelial repair process.

At 5 days postischemia, NCAM is abundant in proximal tubule S3 segments, the nephron segment where damage is most pronounced and cell proliferation most prevalent at earlier times postischemia (45). BrdU labeling of proximal tubules confirmed our earlier findings (45) that most surviving cells in S3 segments enter the cell cycle by 2 days postischemia. These surviving tubular epithelial cells lose their highly specialized phenotype, as reflected by a loss of the apical markers, gp330 and DPP-IV. By 2 days postischemia, S3 segment cells express vimentin, a reflection of increased expression of mesenchymal features (45). The later induction of NCAM at 5 days postischemia may be due to a time delay between induction of gene expression and expression of sufficient protein for detection. Small amounts of NCAM were found in a few cells in S1-S2 segments at this time. Proximal tubule
S1 and S2 segments are less vulnerable to ischemia in this model, and only scattered mitotically active cells were seen in cortical segments. In contrast to the S1 and S2 segments where there is little cell desquamation, many S3 cells slough off the basement membrane after ischemia. The remaining S3 cells are more mitotically active perhaps due to enhanced matrix-cell interactions or lack of inhibitory influences from adjacent cells. S3 cells spread and crawl to supply the necrotic tubule with a new epithelial lining. The need for a prolonged phase of proliferation to repair the epithelium may explain the maintenance of the dedifferentiated phenotype after mitogenesis as reflected by detection of abundant NCAM at 5 days after reperfusion.

Reconstitution of the apical brush border is a key event in the epithelial repair phase (39, 41). There is an inverse correlation between NCAM expression and the presence of specific proteins on the brush border. Even within an individual tubular profile, cells displaying abundant NCAM at their basal and lateral aspects did not express gp330 or DPP-IV apically. A small amount of basolateral NCAM staining was observed on some cells displaying a rudimentary brush border. It is possible that NCAM is removed from basolateral cell membranes as the apical membrane domain is reassembled.

The expression of a number of molecules is tightly coordinated with major morphogenetic events in nephron development. During conversion of mesenchyme to epithelium, NCAM remains for some time at the basal and lateral borders of cells in the condensation phase, when cells manufacture extracellular matrix proteins and uvomorulin (E-cadherin) and when adhesion is important for increasing cell density and cell-cell communication (26, 38, 40). Cell-matrix interactions are known to provide spatial cues for the genesis of epithelial cell polarity (13). At later stages of cell development, NCAM disappears from cell borders. Clues concerning the role of NCAM can be derived from the recognition and signaling functions of NCAM. These include binding (heparin/HSPG and collagen) (10), regulation of matrix metalloproteinases (15), and clustering of fibroblast growth factor (FGF) receptors at contact sites enriched with NCAM (44). Each of these effects of NCAM could be important in the maturation and remodeling of renal tubules (9).

There are molecular and cytoarchitectural analogies between neural cells and polarized epithelia (25, 43), including proximal tubular cells (36). Cell signaling via NCAM that leads to growth of neuronal processes has been linked to the FGF receptor (44), and FGF-1 is expressed in poorly differentiated vimentin-positive regenerative S3 cells after injury to the proximal tubule (23). Both in the developing nephron and recovering tubules, NCAM is synthesized and localized to lateral and basal sides of the cells that express vimentin (45) and that do not display epithelial gp330 and DPP-IV. In both the developing and repairing tubule, NCAM disappears when the brush border assembles. The expression of NCAM and vimentin, with no gp330 and DPP-IV, strongly suggests that the regenerative tubule cells revert back to a developmental equivalent of the renal interstitium or early condensate.

The upregulated NCAM expression in cells of the renal interstitium postischemia may reflect a less differentiated interstitium that will more effectively contribute to morphogenetic programs of the kidney. NCAM, when expressed in fibroblasts, will potentiate the outgrowth of neurites from cocultured neurons, and NCAM has been implicated in neural plasticity associated with memory and learning in the central nervous system (42). The upregulation of NCAM on epithelial cells of recovering proximal tubules probably allows the cells to respond to matrix molecules that trigger mitogenic and motogenic responses recapitulating what occurs in renal development. Mesenchymal-epithelial interactions influence kidney tubulogenesis, through diffusible factors such as hepatocyte growth factor (HGF), members of the transforming growth factor-β (TGF-β) superfamily (which include bone morphogenetic
proteins), members of the Wnt family of signaling proteins, and glial-cell-line derived neurotrophic factor (GDNF) (17, 33). In addition, however, the extracellular matrix is critically important for tubulogenesis, and cells interact with extracellular matrix molecules through molecules of the NCAM immunoglobulin superfamily. NCAM is expressed in flattened cells of a few tubules up to 7 wk after ischemia. These NCAM-positive tubules are almost devoid of apical differentiation markers. This suggests that NCAM expression must be turned off for the process of differentiation and complete restoration of the normal functional phenotype to occur.

Finally, growth factors have been shown experimentally to accelerate postischemic recovery, increasing the rate of renal DNA synthesis (21). Growth factors induced during the postischemic period may be responsible for upregulation of NCAM expression. In neural cells, the synthesis of NCAM and L1, an NCAM-related morphogenetic protein, are induced with osteogenic cells, the synthesis of NCAM and L1, an NCAM-related molecule (NCAM), but not glycosyl-phosphatidylinositol-anchored NCAM, down-regulates secretion of metalloproteinases. Obrink, and E. Bock. Developmentally regulated conversion of mesenchyme to epithelium. J. Cell Biol. 114: 143–154, 1991.


