Proliferative Activity of Intrinsic Cell Populations in the Normal Human Kidney[1]

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ABSTRACT

The proliferative activity of various normal human renal cell populations is unknown. Recently, antibodies to cell proliferation-associated nuclear proteins, such as proliferating cell nuclear antigen (PCNA) and Ki-67, which are applicable to archival paraffin sections, became available. With antibodies to PCNA and Ki-67 after microwave pretreatment of the paraffin sections, the proliferation indexes (ratio of positive nuclei with PCNA and Ki-67 antibodies/all nuclei counted × 100, *i.e.*, percentage of positive cells) of 12 different intrinsic renal cell populations in 20 normal human kidneys have been determined. The following proliferation indexes (percentages of positive cells) were found with the PCNA and the Ki-67 antibodies, respectively: proximal tubular epithelium, 0.22, 0.24; thin limb of Henle, 0.29, 0.30; thick ascending limb of Henle, 0.32, 0.29; distal tubular epithelium (distal convoluted tubules and cortical collecting ducts), 0.33, 0.44; medullary collecting ducts, 0.32, 0.3; glomerular mesangial cells, 0.07, 0.12; glomerular visceral epithelial cells, 0.04, 0.08; glomerular parietal epithelial cells, 0.07, 0.1; glomerular capillary endothelium, 0.42, 0.47; peritubular capillary endothelial cells, 0.38, 0.43; endothelium of large intrarenal vessels (arteries and veins), 0.09, 0.12. Thus, normally capillary endothelium (glomerular and peritubular) appears to have the highest proliferation index in the human kidney by these techniques. These results indicate major variation in the proliferative activity of normal human renal cell populations, along with a significant correlation between PCNA and Ki-67 staining. Furthermore, this study provides normal values for the proliferative activity of different human renal cell populations. Our results can serve as reference values for subsequent studies on various pathologic renal changes, where variations of proliferation indexes from normal may reflect the level of disease activity.

Key Words: *Proliferating cell nuclear antigen, Ki-67, nephron segments, glomerular cells, endothelial cells*

Cell proliferation can be measured by a variety of techniques, including mitotic figure counting, metaphase arrest, incorporation of modified nucleotides (*e.g.*, tritiated thymidine) into newly synthesized DNA, flow cytometric analysis, and silver staining of the nucleolar organizer regions (1). All of these methodologies have advantages and disadvantages; some are applicable primarily to *in vitro* and experimental studies, and others are not well standardized or reproducible.

The proliferative activity of human tissues *in vivo* is difficult to determine. The most commonly used method is counting the mitotic figures in tissue sections. However, the mitotic (M) phase is the shortest phase of the cell cycle; thus, the number of mitotic figures in a tissue section represents only a small portion of cycling cells. In addition, the size of a biopsy specimen may be a further limiting factor in sections of small biopsies (as in most cases of renal biopsies), where frequently, no mitotic figures are seen. Also, mitotic cells cannot always be readily distinguished from pyknotic nuclei. Counting silver-stained nucleolar organizer regions (numbers of deposited silver grains above proliferating nuclei) is also applicable to tissue section, but the method is difficult to standardize. Flow cytometry is a well-standardized method; it renders a DNA histogram, but it cannot discriminate between nuclei of various cell populations within a tissue specimen.

Recently, antibodies to cell proliferation–associated antigens, including proliferating cell nuclear antigen (PCNA) and Ki-67, became available. Some of these latter antibodies can be used on archival formalin-fixed, paraffin-embedded tissue sections (2–6). PCNA (cyclin), a 36-kd acidic, nonhistone nuclear polypeptide (an auxiliary protein to DNA polymerase delta), is clearly associated with cell proliferation (7). Ki-67 is a nonhistone nuclear protein that is exclusively detectable in lysates prepared from proliferat-

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ing cells (8). The exact function of Ki-67 is still not known. Initially, using PCNA and Ki-67 antibodies with standard immunohistochemical methods, results were variable, primarily because of variations in fixation. However, by introducing a microwave pretreatment of tissue sections, as described by Shi *et al.* (9), excellent nuclear immunostaining with both antibodies in a variety of archival surgical specimens (including renal tissues) was obtained.

The proliferation index (percentage of proliferating cells) of various cell populations in the normal human kidney has not been characterized. The shedding of renal tubular epithelial cells is continuous even under normal conditions. Prescott (10) estimated a loss of 68,000 to 72,200 tubular epithelial cells per hour in the urine, indicating that a basic level of tubular epithelial cell proliferation (regeneration) is constant. The proliferation index of 0.1% in proximal tubular cells in the normal rat kidney was reported by McCreight and Sulkin in 1959 (11) by counting mitotic figures. In this study, we determined the proliferation indexes of various renal cell populations in tissue sections of normal human archival renal tissues using anti-PCNA and anti-Ki-67 monoclonal antibodies.

METHODS

Paraffin blocks of histologically normal kidneys were collected from 20 patients: (*1*) six cases were cadaver kidneys preserved for transplantation but not transplanted (age range, 18 to 42 yr; mean, 28 yr); (*2*) seven cases were portions of preserved parts of kidneys surgically removed from adult patients (age range, 42 to 69 yr; mean, 52 yr) because of renal cell carcinoma; and (*3*) seven cases were uninvolved parts of pediatric kidneys resected because of Wilms' tumor (age range, 2 to 7 yr; mean, 4.4 yr). All cases were routinely formalin fixed. The paraffin blocks were selected on the basis of the hematoxylin and eosin stains: only blocks showing no pathologic renal parenchymal changes and no tumor tissue were included. Diameters of tumors in the renal cell carcinoma cases ranged between 4 and 14 cm; in the Wilms' tumor cases, they ranged between 2.5 and 11 cm. Although parenchymal displacement and renal vein invasion occurred, a considerable part of the renal parenchyma was free of tumor in all of the selected cases. Ureteral obstruction was not noted in any of the cases. Five conserved, nontransplanted kidneys (from five cadaver donors) were kindly provided by Dr. Jenö Ormos (Szent-Györgyi Albert Medical University, Szeged, Hungary). The remainder of the kidneys were selected from our archival material (between 1985 and 1992).

Immunostaining with the PCNA antibody (DAKO, Carpinteria, CA) was accomplished by a peroxidase-antiperoxidase method (12) with microwave pretreatment, as suggested by Shi *et al.* (9). After deparaffinization and endogenous peroxidase blocking, slides were placed in 10 mM citric acid buffer (pH 6.0) and microwaved in a 700-W microwave oven at 50% power level for 15 min. Because of the evaporation, distilled water was periodically added to maintain buffer levels. After microwaving, slides were cooled for 20 min at room temperature in the buffer. After a phosphate-buffered saline wash, blocking serum (normal horse serum) was applied for 20 min; then, slides were incubated with the anti-PCNA antibody (diluted 1:50) for 60 min at room temperature. After slides were washed in phosphate-buffered saline rabbit anti-mouse immunoglobulins (DAKO) were applied for 20 min, followed by mouse peroxidase-antiperoxidase complex (DAKO). Color was developed with diaminobenzidine (DAB) as chromogen.

Staining with the Ki-67 antibody (AMAC Inc., Westbrook, ME) (diluted 1:50) was performed by an avidin-biotin-peroxidase complex method (13). After the blocking serum and the primary antibodies, sections were incubated with a biotinylated horse anti-mouse antibody (Vector, Burlingame, CA), followed by the addition of avidin-biotin-peroxidase complex (Vector). DAB was used as chromogen.

Double-labeling studies were performed with four combinations of antibodies: (*1*) PCNA and Tamm-Horsfall protein (THP) (Dr. John Hoyer, Children's Hospital, Philadelphia, PA); (*2*) PCNA and leukocyte common antigen (LCA) (DAKO); (*3*) Ki-67 and THP; and (*4*) Ki-67 and LCA. After the immunostainings for PCNA or Ki-67 were completed, the second immunoreactions with the antibodies to LCA (antibody diluted 1:50) or THP (antibody diluted 1:500) were performed with a streptavidin-conjugated alkaline phosphatase–based system, as described previously (14). The secondary antibody was biotinylated horse anti-mouse (Vector) for LCA staining or biotinylated swine anti-rabbit (DAKO) for THP staining (the antibody to THP that was used was a rabbit polyclonal antibody). The streptavidin-conjugated alkaline phosphatase (DAKO) was applied in a dilution of 1:100. Fast red TR (Biogenex, San Ramon, CA) was used as chromogen. Sections were counterstained with periodic acid–Schiff (PAS).

Evaluation of Slides

Only nuclei and cells showing a strong image/background (signal-to-noise) ratio were counted as positive. Different segments of the nephron were evaluated and manually counted. Proximal tubules, distal tubules, collecting ducts, and the thin limb of Henle were readily distinguished by the PAS stain in light microscopic sections. Proximal tubular epithelial cells have a prominent PAS-positive brush border,

whereas distal tubular epithelial cells are cuboidal, are more densely packed than proximal tubular epithelial cells, and do not have a brush border. We did not distinguish between distal convoluted tubules and cortical collecting ducts; both were included under the term of *distal tubule*. The thin limb of Henle, located in the renal medulla, is a narrow tubule lined with a thin epithelium; accordingly, it can be readily separated from the medullary collecting ducts. In this study, the term *collecting duct* refers to the medullary collecting ducts. The thick ascending limb of Henle is difficult to distinguish by routine staining methods, but it can easily be identified with THP immunostain (this and the very initial portion of the distal tubule are the only nephron segments expressing this protein under normal conditions). In the glomeruli, parietal and visceral epithelial cells, mesangial cells, and endothelial cells were evaluated. On the basis of the PAS reaction, parietal and visceral epithelial cells can be readily identified and separated. Cell nuclei were counted as mesangial if they were embedded within the PAS-positive mesangial matrix. Endothelial cell nuclei line or bulge into the glomerular capillary lumina and are covered only with a thin layer of LCA-negative cytoplasm. Positive immunostaining with the antibody to LCA helped to distinguish glomerular intracapillary leukocytes from endothelial cells. Nuclei of peritubular capillary endothelial cells and endothelial cells lining larger intrarenal vessels were counted separately. Under the term *larger intrarenal vessels*, we included interlobular and larger intrarenal arteries as well as intrarenal veins. We did not separate these different larger blood vessels (*1*) in order to have a large enough population of endothelial cell nuclei to count (*i.e.*, close to 2,000 large vessel endothelial cell nuclei per section), and (*2*) because these endothelial cells had a relatively low proliferation index that did not appear to differ between the various sizes of vessels. Nuclei of smooth muscle cells in the media of interlobular and larger arteries were also evaluated. At least 2,000 nuclei were counted manually in each renal cell population in each case with both the PCNA and the Ki-67 antibodies. The proliferation index (ratio of positive nuclei/all counted nuclei × 100, *i.e.*, percentage of the positive nuclei) for the different cell populations was then determined.

Statistics: Database and Spreadsheet Analysis

All laboratory data were keyed into a database (DBASE III plus; Ashton-Tate, Torrance, CA) program run on a IBM PC/AT (IBM, Valhalla, NY) with 640 Kbytes of RAM memory and equipped with a plotter (Hewlett-Packard, Boise, ID). For correlation studies and x-y line graphs, we ported subsets of this database into Lotus 1–2–3 Release 2.01 (Lotus Development Corp., Cambridge, MA), a spreadsheet program.

In addition to Lotus, we used another software package (Statgraphics Statistical Graphics System, Rockville, MD) for statistical analyses and graphics. For simple linear regression, we used the Pearson product-moment correlation procedure; all variance results were obtained by use of the one-way analysis of variance procedure. Values for y-intercept and slope for each linear curve fit were calculated, as were the correlation coefficient (r), the t distribution, the F-ratio, the probability level (P), and the standard error of regression (Sxy). Statistical comparison between the three different subpopulations (pediatric, adult, and not transplanted kidneys) and the 12 different anatomic sites was not performed, because that would have meant a 12×12×3 comparison matrix.

RESULTS

The immunostaining showed a strong signal/noise ratio, and nuclei were usually strongly positive or totally negative with the Ki-67 antibody. The PCNA antibody gave a somewhat different staining pattern: there were strongly positive (dark brown with DAB) nuclei, but many nuclei showed a mild or moderate intermediate staining. The percentage of these intermediately staining nuclei was approximately 5% in the nuclei of renal tubular epithelial cell populations and in the arterial smooth muscle cells. They were much less apparent in the glomerular and endothelial cell populations. These intermediately staining nuclei were not counted as true positives; only the strongly stained nuclei were included in our statistical evaluations. The positive nuclei were not evenly distributed in the tissue sections. Occasional renal tubular cross-sections showed several positively stained nuclei, whereas in the surrounding tubules, no staining was noted (Figure 1). Mitotic figures were rarely seen. The staining in mitotic cells with the Ki-

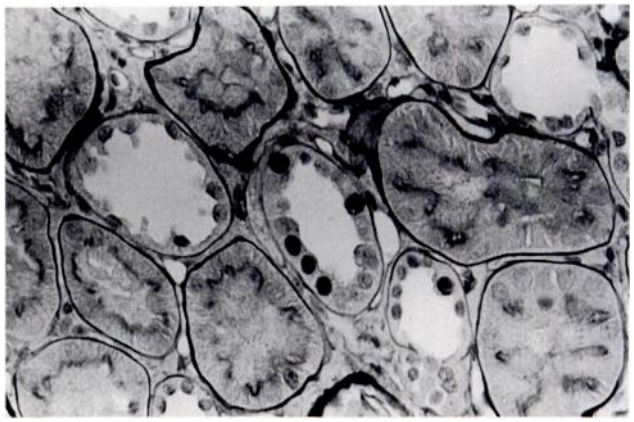

Figure 1. Several PCNA-positive (black) nuclei in a distal tubule. Note that the surrounding tubules do not contain positive nuclei. PAS counterstain, ×400.

67 and the PCNA antibodies was different; the Ki-67 antibody strongly stained the metaphase chromosomes, whereas the PCNA antibody demonstrated a mild to moderate diffuse cytoplasmic staining with no or only faint staining of the chromosomes (Figure 2).

Proliferation Indexes of the Various Renal Cell Populations

In the renal tubular system, the distal convoluted tubules are the most proliferative sites. Glomerular and peritubular capillary endothelial cells also show a high proliferative activity compared with other normal renal cell populations (Figures 1 and 3 to 5; Table 1). Ki-67 positively predominated in these sites, particularly in the distal tubules. Only in vascular smooth muscle cells did PCNA-positive nuclei significantly outnumber Ki-67-positive nuclei. The pediatric kidneys showed a higher proliferation index than did the two adult groups (*i.e.*, adult native kidneys and preserved but not transplanted kidneys) in the renal tubular system, except in the collecting

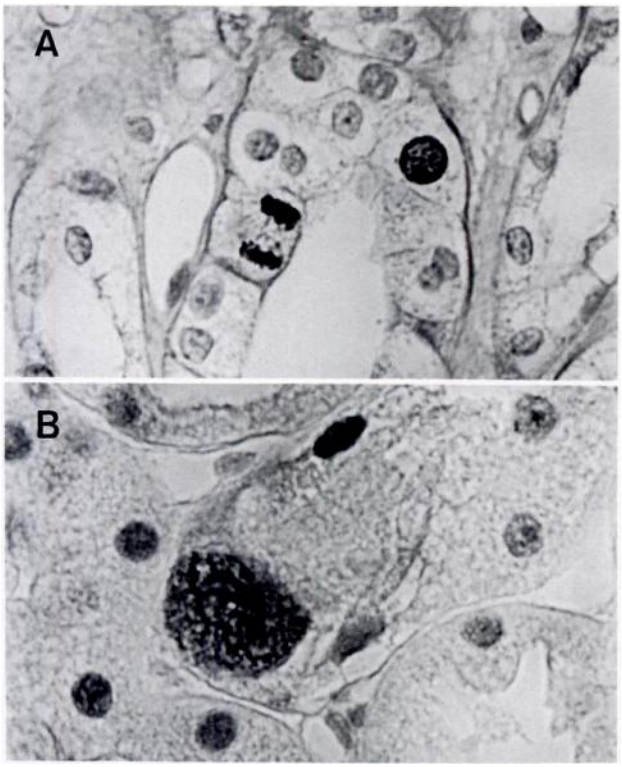

Figure 2. Different staining patterns with the PCNA and Ki-67 antibodies in mitotic (metaphase) tubular epithelial cells. (A) The Ki-67 antibody strongly stains the chromosomes, whereas the cytoplasm is negative. (B) The chromosome staining with the PCNA antibody is mostly masked by a diffuse cytoplasmic staining in this metaphase cell. Magnification, ×1,000.

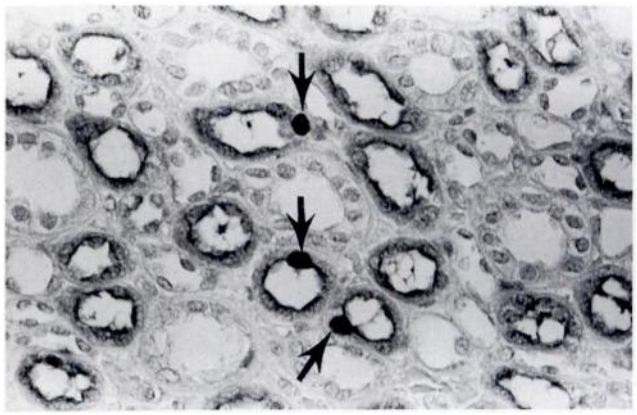

Figure 3. Ki-67-positive nuclei (arrows) in thick ascending limbs of Henle. The tubules with the dark cytoplasm are thick ascending limbs immunostained with the Tamm-Horsfall protein antibody. Magnification, ×200.

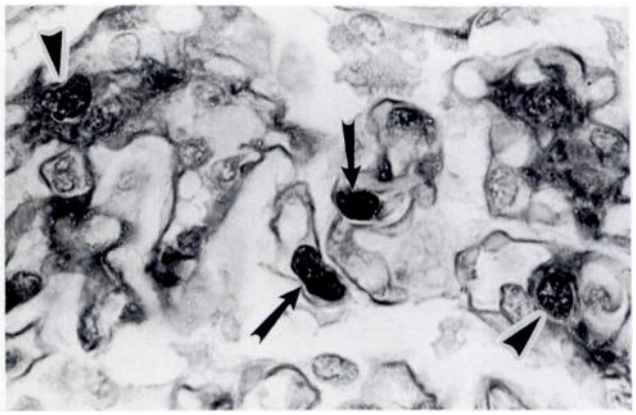

Figure 4. PCNA-positive glomerular endothelial cells (arrows) and mesangial cells (arrowheads). PAS counterstain, ×1,000.

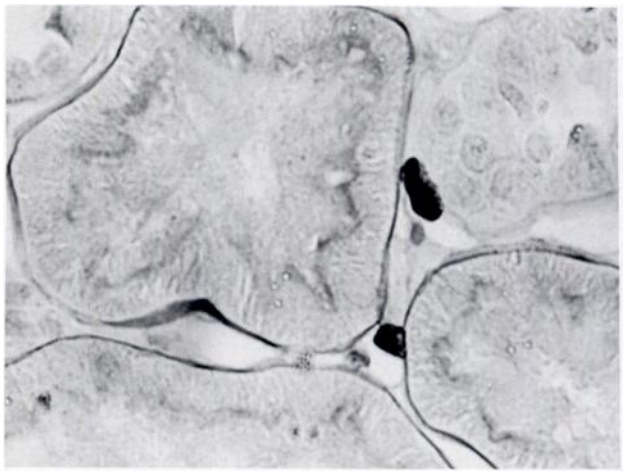

Figure 5. Two Ki-67-positive endothelial cells in peritubular capillaries. PAS counterstain, ×600.

ducts. Very little positivity was noted for either marker in glomerular mesangial cells, glomerular visceral cells (podocytes), and parietal epithelial cells, in arterial smooth muscle cells, and in the endothelial cells of large intrarenal vessels.

Means and Ranges

All cell counting data are shown in Table 1. As can be seen, the means of proliferation indexes for the 240 separate cell populations counted (the mean proliferation index for all cells ± standard deviation) for PCNA and Ki-67 were 0.23 ± 0.26 and 0.24 ± 0.27%, respectively. The maximum value for PCNA and Ki-67 positivity was 1.61 and 1.38%, respectively, both observed in the collecting ducts of the same adult kidney. Minimum values for positivity were 0% for both markers (in occasional cases, no vascular smooth muscle cell nuclei stained).

Correlation Studies

Figure 6 depicts the correlation between the results with the PCNA and Ki-67 antibodies. The slope of the regression line is 0.76 with a y-intercept of 0.07. The standard error of the estimate was ±0.18. A correlation coefficient of 0.71 was calculated and was highly statistically significant ($t = 15.7$; $p < 0.001$). The observed bias between the two markers was not statistically significant. Analysis of variance also showed no significant difference between the two markers when proliferative indexes were compared. There is a scatter about the regression line, as indicated by the relatively high standard error of the estimate, Sxy. However, considering the relatively low number of positive cells observed for most samples, this scatter was not unanticipated.

DISCUSSION

The proliferation rate of various cell populations in the human kidney has not been well characterized. Szabolcs *et al.* (15), in a recent abstract, describe proliferation indexes in various human glomerular diseases with the same antibodies we used, and they also give normal reference values for glomerular endocapillary cells (0.3%), glomerular visceral epithelial cells (0.4%), and parietal epithelial cells (0%) of the Bowman's capsule. The proliferation index for other normal renal cell populations, however, is not indicated (15).

The antibodies to Ki-67 and PCNA that we used react with two different molecules. Most of the PCNA-positive cells are in the S phase, and the Ki-67 positive cells are in the G_2 or M phase of the cell cycle (16–18). Our data confirm these findings because the Ki-67 antibody showed a strong chromosome-associated granular staining in the mitotic cells, whereas with the PCNA antibody, a mild to moderate diffuse cytoplasmic staining with only slight chromosomal staining was noted.

Although the positive correlation between the results with the two antibodies is significant, differences do occur. These differences may be because of (*1*) the differential expressions of the two molecules; (*2*) the subjective errors in estimating the staining intensity (primarily with the PCNA antibody); and (*3*) the occasional clustering of PCNA- or Ki-67-positive cells, particularly in tubular epithelium. This clustering of PCNA- or Ki-67-positive nuclei may reflect paracrine effects in the regulation of renal tubular epithelial cell regeneration. Although the PCNA antibody clearly gives a wider range of staining intensity than does the Ki-67 antibody, similar numbers of proliferating cells were noted if the number of Ki-67-positive nuclei were compared with the number of only the strongly PCNA-positive nuclei.

As anticipated, with the exception of glomerular endothelial cells, glomerular cell populations have a considerably lower proliferation index than do tubular epithelial cells. Glomerular and peritubular capillary endothelial cells showed the highest proliferation rate of all renal cell populations with both markers. Obviously, endothelium can readily regenerate; however, we did not find a reference value in the literature on the normal proliferation of human endothelial cells *in vivo*. Interestingly, the proliferation index of endothelial cells in the larger intrarenal vessels was much lower than in the renal capillaries (glomerular and peritubular). This finding is another confirmation of the heterogeneity of the vascular endothelium (19). It is unclear as to why the proliferation index of the arterial smooth muscle cells is considerably higher with PCNA than with Ki-67. One explanation could be that these cells have a prolonged S phase.

The higher proliferation index of the distal nephron segments (including thick ascending limb of Henle, distal convoluted tubule, and collecting duct) compared with the proliferation index of the proximal tubules is somewhat surprising, and we do not have a good explanation for it. The higher proliferation rate of the tubular epithelial cells in the pediatric kidneys is expected in the growing organ. However, the possibility that growth or proliferation factors might have been released or activated by the adjacent Wilms' tumor in these pediatric kidneys cannot be excluded.

It could be argued that our renal tissues are not necessarily representative of true normal kidneys because many of them were removed because of malignant neoplasms. The surgically removed kidneys from brain-dead patients approximate most closely a normal kidney. However, before formalin fixation, these kidneys underwent preservation pro-

TABLE 1. Summary of data on the cell counts evaluated in the various normal renal cell populations

A: Tubular epithelial cell populations

	ALL				PCT				HEN				TALH				DST				MCD			
	T	A	P	NTX	T	A	P	NTX	T	A	P	NTX	T	A	P	NTX	T	A	P	NTX	T	A	P	NTX
N =	240	84	84	72	20	7	7	6	20	7	7	6	20	7	7	6	20	7	7	6	20	7	7	6
PCNA T	2062	2106	2041	2037	2163	2123	2133	2244	2205	2362	2139	2098	2202	2255	2141	2211	2211	2147	2166	2278	2277	2259	2158	2202
PCNA P	5	5	6	3	5	3	6	6	6	4	9	6	7	4	12	5	7	4	12	5	9	14	8	4
PCNA Pi	0.23	0.21	0.29	0.17	0.22	0.12	0.27	0.26	0.29	0.16	0.43	0.29	0.32	0.18	0.54	0.24	0.33	0.17	0.17	0.24	0.32	0.39	0.39	0.16
PCNA SD	0.26	0.25	0.28	0.21	0.20	0.08	0.21	0.24	0.23	0.07	0.26	0.24	0.32	0.08	0.40	0.22	0.29	0.06	0.30	0.27	0.34	0.51	0.17	0.11
KI-67 T	2057	2058	2081	2028	2157	2237	2124	2101	2087	2096	2048	2123	2100	2033	2023	2268	2123	2091	2133	2149	2189	2404	2059	2089
KI-67 P	5	6	6	3	5	4	8	4	6	5	7	6	6	7	7	3	9	8	14	6	8	11	7	4
KI-67 Pi	0.24	0.26	0.29	0.17	0.23	0.17	0.36	0.16	0.30	0.26	0.35	0.27	0.29	0.34	0.36	0.16	0.44	0.37	0.64	0.29	0.30	0.33	0.34	0.20
KI-67 SD	0.27	0.29	0.27	0.22	0.18	0.15	0.19	0.14	0.20	0.16	0.22	0.21	0.29	0.27	0.31	0.22	0.32	0.15	0.27	0.39	0.32	0.43	0.28	0.10

B: Vascular cell populations

	PCE				LVE				A SM			
	T	A	P	NTX	T	A	P	NTX	T	A	P	NTX
N =	20	7	7	6	20	7	7	6	20	7	7	6
PCNA T	2007	2000	2019	2000	1596	1786	1591	1380	2088	2082	2146	2027
PCNA P	8	11	8	4	2	3	1	0	3	2	4	4
PCNA Pi	0.38	0.53	0.39	0.18	0.09	0.16	0.09	0.01	0.16	0.12	0.17	0.21
PCNA SD	0.24	0.25	0.20	0.13	0.12	0.16	0.04	0.02	0.19	0.08	0.17	0.28
KI-67 T	2096	2000	2218	2065	1723	1817	1831	1487	2043	2020	2059	2050
KI-67 P	9	11	12	4	2	2	3	1	1	1	1	1
KI-67 Pi	0.43	0.56	0.51	0.18	0.12	0.13	0.15	0.07	0.05	0.03	0.06	0.04
KI-67 SD	0 39	0 50	0.32	0.08	0.08	0.08	0.07	0.05	0.05	0.05	0.06	0.02

C: Glomerular cell populations

	GCE				MES				POD				BOW			
	T	A	P	NTX	T	A	P	NTX	T	A	P	NTX	T	A	P	NTX
N =	20	7	7	6	20	7	7	6	20	7	7	6	20	7	7	6
PCNA T	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000
PCNA P	8	10	8	6	1	2	2	1	1	1	1	1	1	2	1	1
PCNA Pi	0.42	0.51	0.42	0.32	0.07	0.09	0.09	0.04	0.04	0.04	0.04	0.04	0.07	0.11	0.06	0.03
PCNA SD	0.26	0.25	0.28	0.17	0.06	0.06	0.06	0.06	0.03	0.32	0.04	0.02	0.09	0.11	0.07	0.05
KI-67 T	2044	2000	2127	2000	2029	2000	2083	2000	2049	2000	2141	2000	2042	2000	2119	2000
KI-67 P	10	11	9	9	2	3	3	2	2	1	2	1	2	3	2	1
KI-67 Pi	0.47	0.54	0.40	0.47	0.12	0.14	0.13	0.10	0.08	0.07	0.09	0.07	0.10	0.16	0.08	0.05
KI-67 SD	0 32	0.31	0.24	0.37	0.11	0.08	0.14	0.10	0.07	0.07	0.06	0.05	0.08	0.09	0.06	0.04

ALL = All anatomic sites together
PCT = Proximal convoluted tubule
HEN = Thin limb of Henle
TALH = Thick ascending limb of Henle
DST = Distal tubule
MCD = Medullary collecting duct
GCE = Glomerular capillary endothelium
PCE = Peritubular capillary endothelium
LVE = Large vessel endothelium
MES = Mesangial cells
POD = Podocytes
BOW = Bowman's capsule epithelium
A SM = Arterial smooth muscle cells

T = Total (all 20 cases)
A = Adult
P = Pediatric
NTX = Not transplanted

PCNA T = Mean of cell nuclei counted
PCNA P = Mean of PCNA positive nuclei
PCNA Pi = Mean proliferation index with the PCNA Antibody
PCNA SD = Proliferation index standard deviation

KI-67 T = Mean of cells counted
KI-67 P = Mean of Ki-67 positive nuclei
KI-67 Pi = Mean proliferation index nuclei with the Ki-67 antibody
KI-67 SD = Proliferation index standard deviation

N = number of cell populations studied

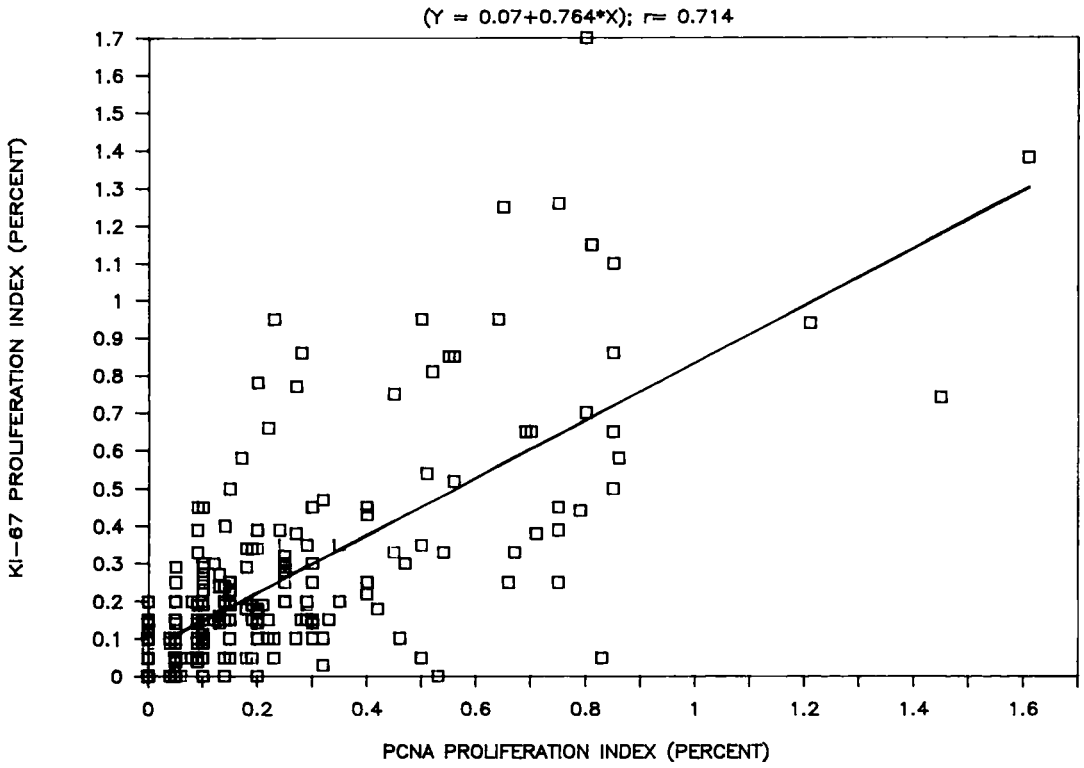

Figure 6. Correlation between proliferation indexes as defined by the Ki-67 and PCNA antibodies.

cedures for transplantation purposes, and it is unclear how this might have influenced the proliferation activity of the various renal cell populations. Of course, totally "normal" human kidneys are essentially impossible to collect. Renal biopsies are not performed on normal kidneys. Moreover, biopsy specimens are often too small to be representative for all renal cell populations. We excluded autopsy kidneys in order to minimize the possible interference of autolysis with the examined protein markers of cell proliferation.

The immunohistochemical method with microwave pretreatment (9) was found to be excellent for the detection of both PCNA and Ki-67 in archival, routinely formalin-fixed, paraffin-embedded tissues. In fact, by this method, superb results can be achieved even after prolonged (even over 2 yr) fixations (20). The age of the specimen does not appear to influence results, either; the method can be successfully applied to sections obtained from paraffin blocks stored for over 60 yr (6).

In summary, we have found that the proliferative activity of various human renal cell populations can be readily determined with anti-PCNA and anti-Ki-67 antibodies, even in routinely formalin-fixed, paraffin-embedded archival material after preincubation of the paraffin sections in a microwave oven. Our study reveals substantial differences in the proliferative activity of the various normal renal cell populations. Furthermore, this study provides the first baseline normal reference values for the subsequent measurement of proliferative activity of different human renal cell populations under pathologic conditions, where the deviation of proliferation indexes from normal may well reflect the presence and degree of disease activity.

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