De novo expression of podocyte proteins in parietal epithelial cells in experimental aging nephropathy

Jiong Zhang,1 Kim M. Hansen,2,3,4 Jeffrey W. Pippin,1 Alice M. Chang,1 Yoshinori Taniguchi,1 Ronald D. Krofft,1 Scott G. Pickering,1 Zhi-Hong Liu,5 Christine K. Abrass,2,3,4 and Stuart J. Shankland1

1Division of Nephrology, Department of Medicine, University of Washington School of Medicine, Seattle; 2Primary and Specialty Care Medicine, Department of Veterans Affairs Puget Sound Health Care System, Seattle; 3Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington School of Medicine, Seattle; 4Program on Allergy and Inflammation UW Medicine Lake Union, Seattle, Washington; and 5Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China

Submitted 8 September 2011; accepted in final form 21 November 2011

De novo expression of podocyte proteins in parietal epithelial cells in experimental aging nephropathy. Am J Physiol Renal Physiol 302: F571–F580, 2012. First published November 30, 2011; doi:10.1152/ajprenal.00516.2011.—A progressive decrease in podocyte number underlies the development of glomerulosclerosis and reduced kidney function in aging nephropathy. Recent data suggest that under certain disease states, parietal epithelial cells (PECs) begin to express proteins considered specific to podocytes. To determine whether this phenomenon increases in aging kidneys, 4-, 12-, and 20-mo ad libitum-fed and 20-mo calorie-restricted (CR) rats were studied. Single and double immunostaining were performed with antibodies to the PEC protein paired box gene 2 (PAX2) and tight junction protein claudin-1, the podocyte-specific protein Wilms’ tumor 1 (WT-1), and the proliferating cell protein (Ki-67). ImageJ software measured Bowman’s basement membrane (BBM) length and glomerular tuft area in individual glomeruli from each animal to assess glomerular size. The results showed that in aged ad libitum rats, the decrease in number of podocytes/glomerular tuft area was accompanied by an increase in the number of PECs/BBM length at 12 and 20 mo (P < 0.01 vs. 4 mo). The increase in PEC number was due to proliferation (increase in PAX2/Ki-67 double-positive cells). Aging was accompanied by a progressive increase in the number of glomerular cells double staining for PAX2 and WT-1. In contrast, the control 20-mo-old CR rats had no increase in glomerular size, and podocyte and PEC number were not altered. These results suggest that although the number of PECs and PECs expressing podocyte proteins increase in aging nephropathy, they are likely not sufficient to compensate for the decrease in podocyte number.

Podocytes are terminally differentiated glomerular epithelial cells that are typically unable to proliferate adequately to replace those lost in disease or aging. However, recent data suggest a possible link between parietal epithelial cells (PECs) and podocytes in the possible repair of glomeruli associated with a decrease in podocyte number (20). The evolving paradigm suggests that in certain diseases, a subpopulation of PECs begin to express proteins considered specific for podocytes. Although it is tempting to speculate that the cells expressing both PEC and podocyte proteins might indicate that PECs are “transitioning” or changing into podocytes, this phenomenon has not been fully proven to date. The purpose of the current study was to use a rat model of aging nephropathy to determine whether there was an increase in the number of PECs expressing podocyte proteins in the aging kidney.

Fig. 1. Bowman’s basement membrane (BBM) length and mean glomerular tuft area increase in aged rats. A: glomerular tuft area (mm²) was significantly larger in 12- and 20-mo-aged ad libitum (ad lib) rats compared with 4-mo ad libitum rats. Glomerular tuft area was significantly smaller in 20-mo-old calorie-restricted (CR) rats. There was no statistically (NS) significant difference in glomerular tuft area at 12 and 20 mo. B: BBM length was statistically significantly longer at both 12 and 20 mo in ad libitum rats compared with 4-mo ad libitum rats, but significantly smaller in 20-mo-old CR rats. There was no statistically significant difference in BBM length at 12 and 20 mo (NS).

It is estimated that by 2030, the number of people living in the US aged ≥65 yr will comprise 19.6% (71 million) of the population, compared with 12.4% of the population in 2000 (8, 25). Studies show an increase in the incidence and prevalence of chronic kidney disease and end-stage kidney disease in this aging population (9, 19). Although several kidney changes accompany aging, several groups have focused on a decrease in podocyte number with age (28), because reduced podocyte number underlies the development of glomerulosclerosis and reduced kidney function (12, 14, 15, 17, 27).
MATERIALS AND METHODOLOGY

Animal Studies

These studies were reviewed and approved by the Institutional Animal Care and Use Committee of the VA Puget Sound Health Care System. Male F344 rats that had free access to feed allowing the animal to self-regulate intake (referred to as ad libitum) were obtained at 4 (n = 10), 12 (n = 10), and 20 mo (n = 10) of age from the National Institutes on Aging’s barrier raised colony. F344 rats that were calorically restricted (referred to as CR) from 4 mo of age were obtained at 20 mo of age. CR was initiated at 14 wk of age at 10% restriction, increased to 25% restriction at 15 wk, and to 40% restriction at 16 wk where it is maintained until 20 mo of age. Five animals in each group were examined. Kidneys were harvested from animals at the time of death and processed as described below. Normal male Wistar rats (3 mo) were used as positive and negative control for the immunostaining described below.

Immunohistochemistry Staining

Single staining. To quantitate changes in podocyte and PEC number, indirect immunoperoxidase staining was performed on 4-μm-thick sections of rat kidney biopsies fixed in formalin and embedded in paraffin as we previously reported (7, 20, 21). In brief, paraffin was removed using Histoclear (National Diagnostics, Atlanta, GA), and sections were rehydrated in ethanol. Antigen retrieval was performed by boiling sections in the microwave in 1 mM EDTA, pH 6.0. Endogenous peroxidase activity was quenched with Alkaline Phosphatase/Horseradish Peroxidase Block (BioFX Laboratories, Owings Mills, MD). Nonspecific protein binding was blocked with Background Buster (Accurate Chemical & Scientific, Westbury, NY). After being blocked, tissue sections were incubated overnight at 4°C with the primary antibodies. The following primary antibodies were used in these studies: rabbit anti-rat paired box gene 2 (PAX2) polyclonal antibody (Zymed Laboratories, South San Francisco, CA) and rabbit anti-claudin-1 polyclonal antibody (Zymed Laboratories) were used to identify PECs; rabbit anti-Wilms’ tumor (WT)-1 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was used to identify podocytes; and rabbit anti-Ki-67 monoclonal antibody (Thermo Fisher Scientific, Fremont, CA) was used to identify proliferating cells.

For PAX2 single staining, a rabbit on rodent horseradish peroxidase (HRP)-Polymer kit (Biocare Medical) was used for additional blocking and substitutive secondary antibody according to the manufacturer’s protocol. Staining was visualized with the Vector SG substrate kit giving a blue gray color (Vector).

For WT-1 single staining, a rabbit on rodent AP-Polymer kit (Biocare Medical) was used for additional blocking and substitutive secondary antibody according to the manufacturer’s protocol. Staining was visualized with Warp Red Chromogen Kit, giving a red color (Biocare Medical).

Double immunostaining methods. To identify and quantitate the number of glomerular epithelial cells that express both podocyte and PEC proteins, and to measure the number of proliferating PECs, double staining was performed using two approaches. First, as we previously reported (20), we performed double staining with antibodies to a cytoplasmic protein (claudin-1 for PEC) and a nuclear protein.
(WT-1 for podocytes). Second, because the volume of the PEC cytoplasm is typically very small and is at times difficult to detect, we used a more sensitive method of double staining for two nuclear proteins. Accordingly, double stains were performed for PAX2 (PEC nuclear protein) with antibodies to either WT-1 (nuclear podocyte protein) or Ki-67 (nuclear proliferation marker).

Staining with antibodies to PAX2 or claudin-1 was performed first. The methods for PAX2 staining were identical to the single staining described earlier and were visualized with the Vector SG substrate kit, with positive cells being blue gray color (Vector). Claudin-1 staining was performed with biotin anti-rabbit IgG (H+L; Vector Laboratories) as secondary antibody according to the manufacturer’s protocol. Staining was visualized by diaminobenzidine (DAB), with positive cells having a brown color.

Following SG or DAB substrate color development blocking steps were performed. Because all the antibodies being used were developed in rabbit, an anti-rabbit IgG antibody Fab fragment (Jackson ImmunoResearch, West Grove, PA) was used to saturate all the binding sites created during the first set of staining. In addition, peroxidase activity derived from the first set of staining was also blocked using Alkaline Phosphatase/Horseradish Peroxidase Block (BioFX Laboratories). Next, the second set of staining was performed for Ki-67 or WT-1. For Ki-67 double staining, a Polink-2 AP Broad detection system (Golden Bridge International, Mukilteo, WA) was used for additional blocking and substitutive secondary antibody according to the manufacturer’s protocol. Staining color was developed using Warp Red Chromogen Kit (Biocare Medical). For WT-1 double staining, either a rabbit on rodent AP-Polymer kit (Biocare Medical) or a rabbit on rodent HRP-Polymer kit (Biocare Medical) was used for additional blocking and substitutive secondary antibody according to the manufacturer’s protocol. Staining was visualized with either Warp Red Chromogen Kit, red color (Biocare Medical) or Vector SG substrate kit, blue/gray color (Vector), respectively.

Quantification of Immunostaining and Statistical Analysis

Quantification of positively stained cells was performed on individual animals at each time point using a combination of bright field and fluorescent microscopy. Double-positive cells were identified as follows. The presence of blue/gray color in the nucleus by bright field microscopy indicated positive staining for PAX2. If the same nucleus also showed the presence of red color in the nucleus by fluorescent microscopy, this indicated positive staining for Ki-67 or WT-1, and the cell was then considered a double-positive stained cell. Twenty to fifty glomeruli were evaluated for each animal in a blinded manner. The mean number of glomeruli analyzed was 44 (95% CI: 41–49) per animal. Because of known changes in glomerular size with aging, ImageJ software was used to measure the length of the Bowman’s basement membrane (BBM) and glomerular tuft area according to The ImageJ User Guide (Version 1.44). These measures were then used as denominators for the number of positively stained PECs and podocytes, respectively. Briefly, we used “set scale” dialog to define the spatial scale of the active image so measurement results could be presented in calibrated units (mm). The freehand line selection tool

![Image](https://example.com/image.png)

**Fig. 3.** Parietal epithelial cell number (PAX2 staining) increases in aging nephropathy. A: number of PAX2-positive cells/mm BBM length was significantly higher at 12 and 20 mo in aged ad libitum and 20-mo CR rats compared with 4-mo ad libitum rats. B–E: representative images of PAX2 nuclear staining at ×400 magnification (arrows indicate examples of positive cells along the BBM, blue/gray color; arrowheads indicated examples of PAX2-positive glomerular cells within the glomerular tuft). F: PAX2 staining was not detected when the primary antibody was omitted as a negative control.
was used to draw a line along Bowman’s basement membrane and
to draw around the glomerular tuft. We then used “measure” dialog to
get the results for BBM length (measured in mm) and glomerular tuft
area (measured in mm$^2$) from the calculation of pixel number by
ImageJ. The data were ultimately expressed as the number of single
and double-positive cells in each glomerulus divided by either BBM
length or glomerular tuft area. These data were averaged to determine
the mean values for all the glomeruli in each study group.

For multiple comparisons, ANOVA was used and post hoc analy-
ses were performed with least significant difference test, $P < 0.05$ was
considered significant. All values are expressed as means ± SD.

RESULTS

Glomerular Tuft Area and BBM Length Increase With Aging

The results of the morphometric measures are shown in Fig. 1. Similar to other published studies (1–4, 28, 29), there is an
increase in glomerular tuft area with aging at 12 and 20 mo
(Fig. 1A; $P < 0.01$ vs. 4 mo), consistent with glomerular
hypertrophy. There was no significant increase in glomerular
area between rats aged 12 and 20 mo. There was also a
statistically significant increase in the length of BBM in aging
rats from 12 and 20 mo (P < 0.01 vs. 4 mo); there was no significant difference in BBM length between rats aged 12 and 20 mo (Fig. 1B; P > 0.05). Interestingly, there was in fact a decrease in BBM length in the 20-mo-aged CR diet rats (P < 0.01 vs. 4 mo).

**Podocyte Cell Number Decreases in Aged Rats**

The number of WT-1-positive cells in the glomerular tuft was quantified and calculated in each individual glomerulus. Because glomerular hypertrophy characterizes aging, the calculated glomerular tuft area (mm²) was used as the denominator to report the data as cell number/mm². Figure 2A shows a significant decrease in podocyte number/mm² in tuft in aged ad libitum diet rats at both 12 (P < 0.01 vs. 4 mo) and 20 mo (P < 0.05 vs. 4 mo). In contrast, podocyte number/mm² in tuft increased in 20-mo-aged CR diet rats, compared with 4-mo ad libitum diet rats (P < 0.01), 12 (P < 0.01)-, and 20-mo (P < 0.01) aged ad libitum diet rats. Representative images of WT-1 staining at the same magnification are shown in Fig. 2, B–F, and arrows indicate examples of positive staining (B = 4-mo ad libitum, C = 12-mo ad libitum, D = 20-mo ad libitum, E = 20-mo CR, and F = negative control). As expected, staining was absent when the primary antibody was omitted.

These data show that this model is characterized by an age-associated reduction in podocyte number, and this change was prevented by calorie restricting rats. Furthermore, calorie-restricted rats had no age-associated changes in podocyte cell number or glomerular area, which led to an increase in the number of podocyte/glomerular area.

**PEC Number Increases in Aged Rats**

Reports show that within the glomerulus, PAX2 staining is normally restricted to PECs (30). Accordingly, staining was performed for PAX2 in aged rats to assess PEC number and the quantitation is shown in Fig. 3A using BBM length as the denominator. There was a significant increase in the number of PECs proliferate in aging kidneys.

To determine whether the increase in PEC number in aged rats was due to proliferation, double immunohistochemical staining was performed with the primary antibodies to Ki-67 and PAX2. The results are shown in Fig. 4A. Two interesting observations were made. First, there was a statistically significant increase in the number of PAX2/Ki-67 double-positive staining cells in aged ad libitum diet rats at 20 mo (P < 0.05 vs. 4 mo, P < 0.05 vs. 12 mo). The double-positive cells were mostly along the BBM. Second, there was no increase in the number of PAX2/Ki-67 double-positive staining cells in aged CR rats at 20 mo, compared with 20-mo-aged ad libitum diet rats (P < 0.01). Representative images are shown in Fig. 4, B–F (B = 4-mo ad libitum, C = 12-mo ad libitum, D = 20-mo ad libitum, E = 20-mo CR, and F = staining controls). Arrowheads indicate either Ki-67 (red)- or PAX2 (blue)-positive staining. Arrows indicate PAX2/Ki-67 double-positive cells. Appropriate controls for the immunostaining showed that these results were not false positives (Fig. 4, F1–4). These data show that PECs proliferate with aging.
Number of Glomerular Cells Staining for Both a PEC and Podocyte Protein Increase in Aging

We previously reported on an increase in glomerular cells expressing both PEC- and podocyte-specific proteins in several animal models characterized by a decrease in podocyte number (20). Given that podocyte number decreases with aging and that the number of PECs increases, we asked whether there is also an increase in the number of glomerular cells expressing both a PEC and a podocyte protein. Accordingly, we performed double immunohistochemical staining using two approaches, one being two nuclear stains, and one being a nuclear and a cytoplasmic stain.

Staining with the primary antibodies to nuclear PAX2 (PEC protein) and nuclear WT-1 (podocyte protein) is shown in Fig. 5A. There was a statistically significant increase in the number of PAX2/WT-1 double-positive staining cells in glomeruli of...
aged ad libitum rats at 20 mo that was not detected at the earlier time points studied (P < 0.01 vs. 4 mo, P < 0.01 vs. 12 mo). In contrast, there was no increase in the number of PAX2/WT-1 double-positive staining cells in glomeruli of 20-mo-aged CR diet rats compared with aged ad libitum rats at both 12 (P < 0.01) and 20 mo (P < 0.01). Representative images are shown in Fig. 5, B–F (B = 4-mo ad libitum, C = 12-mo ad libitum, D = 20-mo ad libitum, E = 20-mo CR, and F = staining controls). Arrowheads indicate either WT-1 (red)- or PAX2 (blue)-positive staining. Arrows indicate PAX2/WT-1 double-positive cells. Appropriate controls for the immunostaining showed that these results were not false positives (Fig. 5, F1–4). These data support the notion that glomerular cells staining for PEC and podocyte proteins increased in the glomerulus with aging.

The presence of glomerular cells expressing both a PEC and a podocyte protein was further confirmed in the 12- and 20-mo-aged ad libitum rats using antibodies directed to a cytoplasmic (claudin-1, a PEC tight junction protein) and nuclear (WT-1 a podocyte nuclear protein), and representative images are shown in Fig. 6, A–E (A = 4-mo ad libitum, B = 12-mo ad libitum, C = 20-mo ad libitum, D = 20-mo CR, and F = staining controls). Arrowheads indicate either claudin-1 (brown)- or WT-1 (blue)-positive staining. Arrows indicate claudin-1/WT-1 double-positive cells. Appropriate controls for the immunostaining showed that these results were not false positives (Fig. 6, E1–4). These data support the notion the number of glomerular cells expressing both a PEC and a podocyte protein increased with aging.

DISCUSSION

Several characteristic changes have been described in aging kidneys, including widening of the glomerular basement membrane, expansion of the mesangial compartment, enlargement of the glomerulus, and glomerulosclerosis (1, 2, 28, 29). Seminal studies by Wiggins et al. (29) showed decreases in podocyte number in aging were associated with progressive glomerular enlargement and glomerulosclerosis. Moreover, podocytes undergo hypertrophy rather than hyperplasia, which is associated with glomerular enlargement. Several stages of podocyte “adaptation” have been described: hypertrophy alone, hypertrophy accompanied by structural changes but normal function, and finally “decompensation” where hypertrophy is accompanied by changes in key functional proteins with eventual podocyte loss (3, 4, 28, 29). In the current study, we show that PEC number increases in aging kidneys, accompanied by an increase in the number of glomerular cells expressing proteins considered specific for PECs and podocytes.

The current model is used is similar to reports by others showing that podocyte number decreases in aging kidneys. The first finding in the current study was that PECs increase significantly in number in aged kidneys at 12 and 20 mo. The increase was not simply because of an increase in glomerular size, because the calculations used the length of Bowman’s basement membrane as the denominator for PEC number. Moreover, double immunostaining using Ki-67 showed that the increase was indeed due to PEC proliferation, at least at the 20-mo time point. That Ki-67 did not increase at earlier time points may simply reflect sampling error or timing. PEC proliferation and increased number have typically been associated with crescentic glomerulonephritis and collapsing glomerulopathy. The data presented here occurred in the absence of histological evidence of crescent formation. The mechanisms for the increase in PEC proliferation and number were not examined in the current study.

Most reported studies in aging nephropathy have focused on 20- to 30-mo-old rats, where the glomerular basement membrane is thickened, podocytes are significantly reduced in number, and glomerulosclerosis and tubulointerstitial fibrosis

**Fig. 5.** Glomerular cells staining double-positive cells for PAX2 and WT-1 increased in aging nephropathy. A: number of cells staining positive for PAX2 and WT-1/glomerulon was significantly higher at both 12 and 20 mo in ad libitum rats compared with 4-mo ad libitum rats. PAX2/WT-1-positive cells/glm was significantly lower at 20 mo in CR rats compared with all ad libitum rats. There was an increase in PAX2/WT-1-positive cells/glm at 20 mo compared with 12 mo. B–I: low-powered light microscopic view of double staining for PAX2 (nuclear, blue gray) and WT-1 (nuclear, red) in 4-mo ad libitum rats. B: light microscopic view of the inset shown in B–I. The arrowheads indicate examples of a PAX2-positive (WT-1-negative) cell and a WT-1-positive (PAX2-negative) cell. B: fluorescent microscopic view of the inset shown in B–I; only WT-1 staining is seen because only the warp red substrate is visible by fluorescent microscopy. The arrowheads indicate examples of a PAX2-positive (WT-1-negative) cell and a WT-1-positive (PAX2-negative) cell. * Indicates the WT-1-negative nucleus. B–I: combined light and fluorescent microscopic view of the inset shown in B–I, where fluorescent warp red is superimposed on the bright field. The arrowheads indicate PAX2-positive (WT-1-negative) and WT-1-positive (PAX2-negative) cells. C–1: low-powered light microscopic view of double staining for PAX2 (nuclear, blue gray) and WT-1 (nuclear, red) in 12-mo-old ad libitum rats. C–I: light microscopic view of the inset shown in C–I. The arrow indicates an example of a PAX2, WT-1 double-positive cell. C–I: combined light and fluorescent microscopic view of the inset shown in C–I, where fluorescent warp red is superimposed on the bright field. The arrow indicates an example of PAX2, WT-1 double-positive cell. D–I: low-powered light microscopic view of double staining for PAX2 (nuclear, blue gray) and WT-1 (nuclear, red) in 20-mo ad libitum rats. D–I: light microscopic view of the inset shown in D–I. The arrowheads indicate examples of PAX2-positive (WT-1-negative) cells and WT-1-positive (PAX2-negative) cells. The arrow indicates an example of a cell double staining for PAX2 and WT-1. D–I: fluorescent microscopic view of the inset shown in D–I; only WT-1 staining is seen because only the warp red substrate is visible by fluorescent microscopy. The arrowheads indicate examples of PAX2-positive (WT-1-negative) cells and WT-1-positive (PAX2-negative) cells. The arrow indicates an example of a cell double staining for PAX2 and WT-1. D–I: fluorescent microscopic view of the inset shown in D–I; only WT-1 staining is seen because only the warp red substrate is visible by fluorescent microscopy. The arrowheads indicate examples of PAX2-positive (WT-1-negative) cells and WT-1-positive (PAX2-negative) cells. The arrow indicates an example of a cell double staining for PAX2 and WT-1. E–I: fluorescent microscopic view of the inset shown in E–I; only WT-1 staining is seen because only the warp red substrate is visible by fluorescent microscopy. The arrowheads indicate examples of PAX2-positive (WT-1-negative) cells and WT-1-positive (PAX2-negative) cells. The arrow indicates an example of a cell double staining for PAX2 and WT-1. E–I: fluorescent microscopic view of the inset shown in E–I; only WT-1 staining is seen because only the warp red substrate is visible by fluorescent microscopy. The arrowheads indicate examples of PAX2-positive (WT-1-negative) cells and WT-1-positive (PAX2-negative) cells. F: representative images of controls in which primary antibodies were omitted. Images from normal rats, which served as positive control PAX2 and WT-1 (F1–I), PAX2 only (F2–I), WT-1 only (F3–I), and no primary antibody (F4–I). Original magnification X400.
predominate. Thus, this is one of the first studies to examine and find significant age-related changes at 12 mo. These are early middle age animals and clearly a first look at onset harbingers of what is to come (an end-stage kidney). The authors recognize that while the effect of calorie restriction on the preservation of renal function with aging is interesting, it may not be appropriate to evaluate the effect of calorie restriction on the renal function at this time point because the aging ad libitum rats did not have increased blood urea nitrogen compared with young ad libitum rats.

There are increasing data from our group and others showing that in certain forms of glomerular diseases, PECs begin to express proteins that traditionally have been considered specific for podocytes (5, 6, 16, 20, 22–24). A second major finding in this study was that the number of glomerular cells that double stained for WT-1 and PAX2 or claudin-1 and WT-1 increased significantly at 20 mo of age. Although there was a trend toward an increase in transition cells at 12 mo, this was not statistically significant.

Some have coined the term “transition cells” as glomerular cells that express both a PEC and a podocyte protein. To our knowledge, there are no published data at the time of this report showing that transition cells are indeed functional podocytes derived from PECs. Moreover, the mechanisms explaining how PECs begin to simultaneously express podocyte proteins have not yet been elucidated in any disease state, and we do not provide any mechanistic insights with these studies. What is consistent however is that in states characterized by reduced podocyte number, the number of glomerular transition cells increases. We can now add aging to this list. We speculate that a reason for PEC proliferation with aging is to compensate for their transitioning to restore and maintain cell number. Regardless, our data would suggest that the increase in PEC number and the increase in the number of PECs expressing podocyte proteins are insufficient to replace and maintain podocyte number in aging and may explain in part why there is a progressive decrease in podocyte number.

PECs are known to respond to injury by dedifferentiating into an embryonic phenotype, similar to that of myofibroblasts, with de novo expression of α-smooth muscle actin (18). Recently, Swetha and colleagues (26) focused on the relationship between PECs and epithelial-mesenchymal transition (EMT). In their studies, cultured PECs of adult murine kidney underwent EMT and generated cells with characteristics of renal progenitors. Over several passages, the PECs became positive for CD24 with high expression of metanephric mesenchymal markers.ママ

Fig. 6. Glomerular cells double stain for claudin-1 and WT-1 in aging nephropathy. A-I: low-powered light microscopic view of double staining for claudin-1 (cytoplasmic, brown) and WT-1 (nuclear, blue/gray) in 4-mo-old ad libitum rats. A-2: light microscopic view of the inset shown in A-I. The arrowheads indicate examples of a claudin-1-positive (WT-1-negative) cell and WT-1-positive (claudin-1-negative) cells. B-I: low-powered light microscopic view of double staining for claudin-1 (cytoplasmic, brown) and WT-1 (nuclear, blue/gray) in 12-mo-old ad libitum rats. B-2: light microscopic view of the inset shown in B-I. The arrowheads indicate examples of WT-1-positive (claudin-1-negative) cells. The arrow indicates an example of a cell double staining for claudin-1 and WT-1. C-I: low-powered light microscopic view of double staining for claudin-1 (cytoplasmic, brown) and WT-1 (nuclear, blue/gray) in 20-mo-old ad libitum rats. C-2: light microscopic view of the inset shown in C-I. The arrowheads indicate examples of WT-1-positive (claudin-1-negative) cells. The arrow indicates an example of a cell double staining for claudin-1 and WT-1. D-I: low-powered light microscopic view of double staining for claudin-1 (cytoplasmic, brown) and WT-1 (nuclear, blue/gray) in 20-mo-old CR rats. D-2: light microscopic view of the inset shown in D-I. The arrowheads indicate examples of a claudin-1-positive (WT-1-negative) cell and WT-1-positive (claudin-1-negative) cells. E: representative images of controls in which primary antibodies were omitted. Images from normal rats, which served as positive control claudin-1 and WT-1 (E-I), claudin-1 only (E-2), WT-1 only (E-3), and no primary antibody (E-4). Original magnification ×400.
enchymal proteins (Eya-1, Six1 Odd1, Wt-1, and GDNF). Like our study, the cells retained PAX2 expression during their transition. Although these studies focused on PEC to EMT phenotypic changes and our study focused on the expression of podocyte proteins by PECs with age, the plasticity of PECs seems to be a common feature of these cells.

In this study, the effects of a calorie-restricted diet were determined in aged rats. Wiggins et al. (28, 29) used the Fischer 344 rat model of aging and showed an increase in glomerular volume and an expanded mesangial compartment in aged ad libitum-fed older rats compared with calorie-restricted rats. This phenomenon was further validated by measuring ceruloplasmin mRNA expression (28). In their study, the glomerular volume was actually smaller at all time points studied (6, 17, and 24 mo) in calorie-restricted rats. Similarly, in this study there was also no increase in glomerular size and in fact a decrease in both glomerular size and BBM length at 20 mo with calorie restriction. The decrease in glomerular size actually caused an increase in both podocyte and PEC number in this group. The reasons behind the differences between aged ad libitum and CR rats are not fully understood, although high insulin due to higher caloric intake, larger body size, and higher growth factor levels are all suspects (10, 11, 13).

In summary, the absolute number of PECs and the number of PECs expressing both PEC and podocyte proteins increase in aging nephropathy. However, these increases are insufficient to prevent a decrease in podocyte number. More studies are needed to examine the mechanisms underlying these events, and to determine whether glomerulosclerosis can be further reduced by further increasing PECs that begin to express podocyte proteins.

ACKNOWLEDGMENTS AND GRANTS

This material is based on work supported (in part) by the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development, Biomedical Laboratory Research and Development and was supported with resources and the use of facilities at the VA Puget Sound Health Care System, Seattle, WA. These studies were supported by a Merit Review award from the Medical Research Service of the Department of Veterans Affairs (to C. Abrass) and by National Institutes of Health Grants R01DK056799, R21DK081835 (to S. J. Shankland), and R01DK068539 (to C. K. Abrass). This work has been made possible through J. Zhang’s Interna-

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

The contents of this report do not represent the views of the Department of Veterans Affairs or the United States Government.

AUTHOR CONTRIBUTIONS


REFERENCES

22. Ohse T, Pippin JW, Vaughn MR, Brinkkoetter PT, Kroff JD, Shankland SJ. Establishment of conditionally immortalized mouse glo-

