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آموزش مهارت های کاربردی در تدوین و چاپ مقاله
Inductive Role of Collagen Type IV During Nephrogenesis in Mice

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Introduction: During nephrogenesis, transition of mesenchyme to the epithelium of tubules and glomeruli occurs via the interaction of ureteral bud and metanephric mesenchyme. The distribution pattern of collagen type IV suggests that a regulated balance of activities is required to facilitate migration of the ureteral bud branches into the mesenchyme and to control early extracellular matrix changes during tubulogenesis. We used a specific antibody for tracing collagen type IV basement membrane during renal tubules morphogenesis.

Materials and Methods: Twenty female Balb/C mice were divided randomly into 10 groups and were kept until finding vaginal plug was as an indicator of day zero of pregnancy. Twelve pregnant mice were sacrificed by cervical dislocation in one of gestational days 13 to 18 and their fetuses were fixed, serially sectioned, and underwent immunohistochemical study for tracing of collagen type IV in basement membrane of glomeruli. The same processes were used for kidneys preparation on postnatal days 5, 10, 15, and 20 in newborns of 2 mothers for each day.

Results: Collagen type IV showed weak reaction on day 14 of gestation in tubular basement membrane. The amount of collagen increased continuously until the following days of fetal life and of the first 5 postnatal days in basement membrane. After this period, collagen type IV reaction did not show significant change in newborns.

Conclusion: These results indicate that developmental changes in various nephron segments from most immature stages to most differentiated structures are dependent on the collagen type IV expression.

INTRODUCTION

Previous studies have shown that various types of collagen have been found in tissues so far, and different structures of polypeptides are responsible for varieties of collagens.1 Tropocollagen molecules hold together and form collagen fibers.2 The ends of adjacent molecules overlap each other. These arrangement cause stretch and stability for collagen fibers.3 In spite of the fact that other collagens have fibril or reticular structure, collagen type IV has an unpolymerized structure and it is the main component of basement membranes (BMs).4 Although other proteins such as laminin and fibronectin play a crucial role, collagen type IV is the most
abundant composition of the BM and play a key role in formation of the BM.\(^{(5)}\)

During formation of metanephrose, the ureteral bud grows into the metanephric mesenchyme and division of it begins continually, and branches up to collecting tubule form during tubules morphogenesis.\(^{(6)}\) This stage is about embryonic day 11 in mouse (equal to the 5th week in human).\(^{(7)}\) The embryologic studies show that the distal end of collecting tubules causes induction effects on mesenchyme cells of metanephric blastoma and transit to metanephric tubules on the 13th embryonic day.\(^{(8)}\) In this stage, the distal part of tubules forms when the proximal part evaginates by differentiated bowman capsule. During next days of development, different parts of a nephron, consisting of renal corpuscles, proximal convoluted tubule, loop of Henle, and distal convoluted tubules, will develop and associate with collecting tubules.\(^{(9)}\) Some studies have shown that fibroblasts are responsible for producing amino acids that synthesize procollagen molecules.\(^{(10)}\) These molecules alter to tropocollagens by procollagen peptidase enzyme. Polymeric tropocollagens produce collagen and prevent from collagen distribution by hyaloronic acid and help to deposit in BM.\(^{(10)}\) Extracellular matrix and tubular BM contribute to form nephrons during kidney tubule morphogenesis. The present investigation was carried out to demonstrate collagen type IV expression and distribution pattern in nephron morphogenesis and its structural changes from immature nephron to differentiated nephron.

MATERIALS AND METHODS

Study Subjects

Twenty virgin female Balbc/c mice were divided randomly into 10 groups and finding vaginal plug was designated as day zero of pregnancy. Two pregnant mice were anesthetized by chloroform and were sacrificed by cervical dislocation during gestational day 13 to 18. The kidneys of the fetuses were collected and were processed for histological studies. The similar processes were used for newborns on postnatal days 5, 10, 15, and 20. Finally, all samples of fetuses and new borns were placed in paraffin blocks and sectioned serially at a thickness of 7 μm. The study protocol complied with the national ethics regulations for studying on animal models, and was approved by the local ethics committee.

Methods

After deparaffination and rehydration, sections of kidneys were washed twice for 5 minutes with Tris buffer (containing 1.5% sodium chloride at a pH of 7). Nonspecific antibodies were blocked with 3% Triton X-100 and goat serum for 3 hours. For blocking endogenous peroxides activity, the sections were treated with 3% H\(_2\)O\(_2\)-methanol for 1 hour and were incubated with the antibody collagen type IV (conjugated with horse radish peroxidase) at a dilution of 1:50 overnight. Then, the sections were again placed in Tris buffer solution containing 3% Triton and 2% goat serum and were washed three times for 10 minutes with Tris buffer. After this stage, the sections were placed for 15 minutes in Di-aminobenzidine containing 0.03% H\(_2\)O\(_2\), and after washing, the samples were counterstained with hematoxylin. The sections were mounted with glycerol gel.

In this method, collagen would show positive reaction according to the amount of appearance and the rate of reaction, from light to dark brown. Because collagen immunoreaction is a proper index for determination of its density, Firth and Reade’s method was used for grade staining.\(^{(11)}\) This grading was scored ranging from zero to 4+ in conformity with the severity of reaction, corresponding to negative, weak, moderate, strong, and highly strong. Images of different regions of the kidneys were captured by a camera microscope and the intensity of staining was graded by two separate individual according to the above method.\(^{(11)}\)

Statistical Analyses

Statistics analyses of the results were completed by using the SPSS (Statistical Package for the Social Sciences, version 11.5, SPSS Inc, Chicago, Illinois, USA) and data analyses were done using the nonparametric Kruskal-Wallis test and
Mann-Whitney $U$ test. P values less than .05 were considered significant.

RESULTS

Although the mesenchyme cells were enclosed by the ureteral bud in embryonic day 13 and rudimentary tubules were observed, collagen type IV could not be found in any part of the developing nephron. In addition, there was no such reaction in different parts of metanephrose (Figure 1, Left). The first immunostaining was weakly detected around day 14 of gestation in distal tubular BM and vessels in kidney parenchyma, whereas the BMG did not show any reaction (Figure 1, Middle). The intensity of staining increased on day 15 of gestation in the distal tubular BM, proximal tubular BM, and cortical regions of the glomerulus (Figure 1, Right). The amount of collagen increased continuously on day 16 of gestation in different parts of the tubular BM (Figure 2, Left). The intensity of reaction increased gradually until day 18 of gestation not only in the BM of epithelial cells, but also in the tufts of the capillary (Figure 2, Middle). The amount of collagen increased continuously until 5 days postnatal in the BM (Figure 2, Right), but no remarkable change was recorded afterwards in the newborns (Table).

![Figure 2. Sections through kidney tubules on 16, 18, and 5 postnatal days (left to right, respectively). The amount of collagen increased continuously with development of kidney tubules. The reaction changes from light to dark brown are represents the strong staining.](image)

<table>
<thead>
<tr>
<th>Day</th>
<th>Distal Tubular Basement Membrane</th>
<th>Proximal Tubular Basement Membrane</th>
<th>Cortical Regions of Glomerulus</th>
<th>Vessels</th>
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*This gradation was scored ranging from negative to 4+ in conformity with the severity of reaction from negative, weak, moderate, strong, and highly strong.
DISCUSSION

The appearance and high density of collagen type IV during tubules morphogenesis represents that kidney formation is dependent on specific molecules, of which collagen type IV is the most important. Basement membrane is a specialized region of extracellular matrix that consists of different components, such as proteins and carbohydrates. This substance usually comprises from collagen types IV and V, laminin, fibronectin, and sulfated and nonsulfated glycosaminoglycans. Collagen is the most abundant composition of the BM and among its different types, type IV is the main structural component of the BM. Studies have shown that different types of collagen had widespread distribution, whereas collagen type IV was specifically found in the BMs of epithelial tissues such as the endothelium of vessels, gastrointestinal tract, kidney tubules, and glomerulus. On the other hand, although collagen type IV is not distinguished with routine histological staining, immunohistochemistry technique showed that the immune reaction of collagen begins when nephrons structure forms. This confirms that interactions of tubular structures lead to induction of collagen synthesis and help to provide a bed of BM. The appearance of collagen type IV in distal tubular and proximal tubular BM and BM of glomeruli indicates that in addition to serving structural role, collagen plays roles for exchange of substance and glomerular filtration in tubular BM and BM of glomeruli. The composition of collagen type IV in different stages of development is required for proper function of the kidney in filtration and exchange of substances. An intact BM contributes to selective absorption of molecules as a filter. It seems damage to collagen type IV results in functional and structural defects in the kidney. For example, in diabetes mellitus, collagen changes cause thickening of the BM that can affect kidneys’ filtration rate seriously. Our results indicated the amount of collagen did not change after final development of tubules. These data may refer to this fact that if a high density of collagen increases continuously during postnatal days, it may cause thickening of the BM and kidney dysfunction. For example, age-related changes in tissues and hyperglycemia in diabetes mellitus result in a high density of collagen type IV and other components of the BM. Hence, it
seems epithelium arrangement of kidney tubules and proper function of nephrons are dependent upon BM synthesis.

CONCLUSION

Collagen type IV, one of the most remarkable structures of the BM, is synthesized under induction mechanisms and it presents close to the primordial of rudimentary tubules. The first sign of collagen was detected on day 14 of gestation in the BM and increased in next day, which suggests that tubular development is dependent on components of BM formation such as collagen type IV. After birth, when nephrons are developed, collagen density did not show any changes in newborns.

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CONFLICT OF INTEREST

None declared.

REFERENCES


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