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Pattern of collagen IV expression in glomerular and mesenchymal basement membrane during fetal and postnatal period of Balb/c Mice

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Abstract

Basement membrane of glomerular mesangium (BMG) is a thin membrane which helps to support the capillary loops in a renal glomerulus and type IV collagen is require for complete BM formation during glomerulogenesis. In this investigation specific antibody of type IV collagen has been used in light microscopy to study development of BMG of the embryonic and postnatal mouse glomerular mesangium. In this study, 20 female Balb/C mice were selected randomly and finding vaginal plug was assumed as day zero of pregnancy. 12 pregnant mice were sacrified by cervical dislocation in one of gestational days 13-18, their fetuses were fixed and serially sectioned. Immunohistochemical Study for tracing of collagen type IV in BMG was carried out. The same processes were carried out for kidneys preparation of pups on 5, 10, 15 and 20 days after birth (2 mothers for each day). The result of the present study revealed that collagen IV reaction was weak on day 15 of gestation. The amount of collagen increased continuously until next days of fetal life and primary of 10 days postnatal in BMG. After this period, collagen IV showed no significant change in newborns. These data indicate that collagen IV appears just during the glomerular vasculogenesis and because of continuity and glomerular endothelial cell differentiation, type IV collagen, is the major structural protein in BMG, have been implicated in these processes.

Keywords: collagen IV, glomerular basement membrane, kidney, mouse

Introduction

Basement membrane is a specialized structure of extra cellular matrix and consists of different compositions such as proteins and sugars (Gulberg et al., 1995; Berkholtz et al., 2006). This structure contains several components such as types IV, V collagen, laminin, fibronectin, sulfated and nonsulfated glycosaminoglycan (Berkholtz et al. 2006; Mates et al., 2004). Among these compositions, collagen is the most abundant constituent and type IV collagen has been identified a main structure of it (Thesleff et al., 1979; Horacek et al., 2004). Basement membrane not only changes during embryonic period but also alters during later stages of life and its alterations considers as an index of tissue changes in pathologic studies (Poschl et al., 2004; Cosgrove et al., 2008). ECM components turn over continually in developmental organs (Carnegie, 1993; Chai et al., 2003). In other words, molecules and matrix components are required in cell differentiation. Among them, type IV collagen play complex roles in developmental phenomena such as migration, proliferation, morphogenesis and metabolism (Paralkar et al., 1991; Thesleff et al., 1979). The most prominent role of extra cellular matrix is migration and cell adhesion that type IV collagen serves them (12, 13, 14). Therefore, considering collagen plays role in vital organs changes, it necessary seems its investigation.

Material and Methods

20 virgin female Balbc/c mice were selected randomly and finding vaginal plug were designated as day zero of pregnancy. 2 mices were anesthetized by chloroform and were sacrified by cervical dislocation in every gestational days 13-18. Their fetuses were collected and were processed for histological studies. The similar processes were used for newborns on 5, 10, 15 and 20 of postnatal days. Kidneys were isolated from newborns of 2 mothers for each day. Finally, all samples of fetuses and new borns were placed in paraffin blocks and sectioned serially at a thickness of 7 µm. After deparaffination and rehydration, sections of kidneys were washed twice for 5 min with Tris buffer

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(containing 1.5% sodium chloride at PH=7). None-specific antibodies were blocked with 3% Triton X-100 and goat serum for 3 hours. For blocking endogenous peroxides activity, sections were treated with 3% H_2O_2- methanol for 1 hour and were incubated with the monocolonal antibody against collagen IV (conjugated with Horse radish peroxidase) at a dilution 1:50 overnight. Then sections again were placed in Tris buffer solution containing 3%Triton and 2% goat serum and were washed three times for 10 min with Tris buffer. After this stage, sections were placed for 15 min in Di-aminobenzidine containing 0.03% H_2O_2 and after washing samples, were counterstained with hematoxylin. The sections were mounted with glycerol gel. In this method, collagen shows positive reaction according to amount of appearance and the coloring reaction is from light to dark brown. The coloring reaction of collagen is a proper index for determination of BMG. The images of glomerular regions of kidneys were obtained by a camera microscope and were saved as a file. The intensity of collagen IV reaction was graded by two separate individual according to *firth* method (Firth et al., 1996).

Statistical analysis

The data were analyzed by using SPSS software and Kruskal Wallis and Mann-Withney tests. *P*-values<0.05 was considered as significant.

Results

Our finding revealed although mesenchymal cells are enclosed uretric bud and glomerular primordium and rudimentary tubules observed on day 13 of gestation (figure1), no collagen reaction was detected until this period of time. Glomerular development completed on embryonic day 14 and collagen just showed weak reaction in parenchym of vessels (PV) but not in glomerular basement membrane. Collagen IV showed first reaction on day 15 of gestation in cortical glomerulus (figure 2). The intensity of reaction increased continually during next days and detected on day 18 of gestation in basement membrane of cortical glomerulus (figure 2). The results of this stage showed that epithelial basement membrane not only appeared in cortical regions of gelumerulus but also observed in tufts of capillary. The observations on 5, 10 and 15 days of postnatal period indicated that collagen reaction was more intensive on day 5th of postnatal period in glomerular basement membrane (table 1) but this reaction was not increased showed significantly on day 10th and after that during postnatal days.

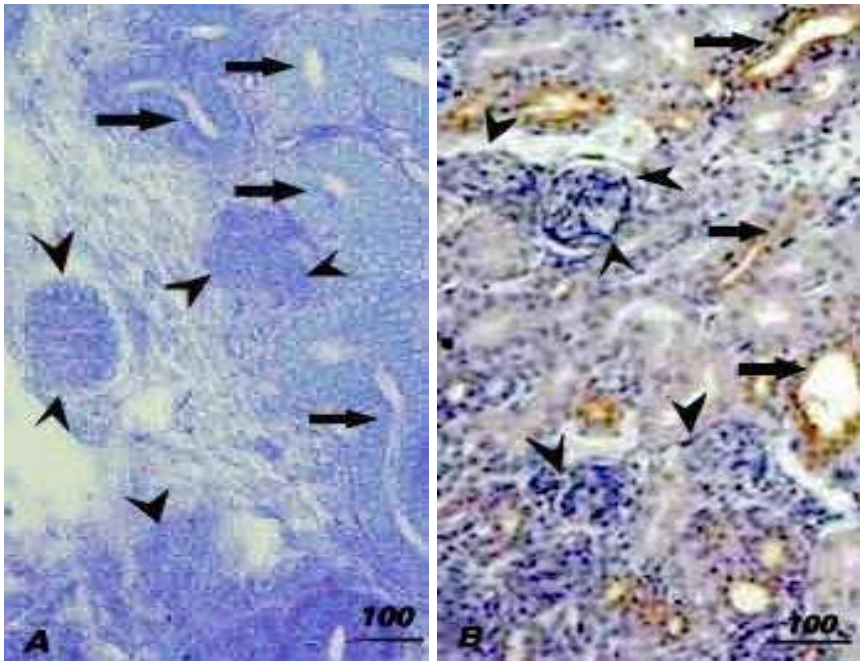

Figure 1. a. Transverse section through kidney paranchyme on day 13 of gestation that collagen indicated no reaction in glomerular primordium (arrow heads) as well as rudimentary tubules (arrows). b. Other transverse section during glomerular development on day 14 of gestation. Although glomerular structure have been completed, no reaction was observed in any area except for parenchyma vessles.

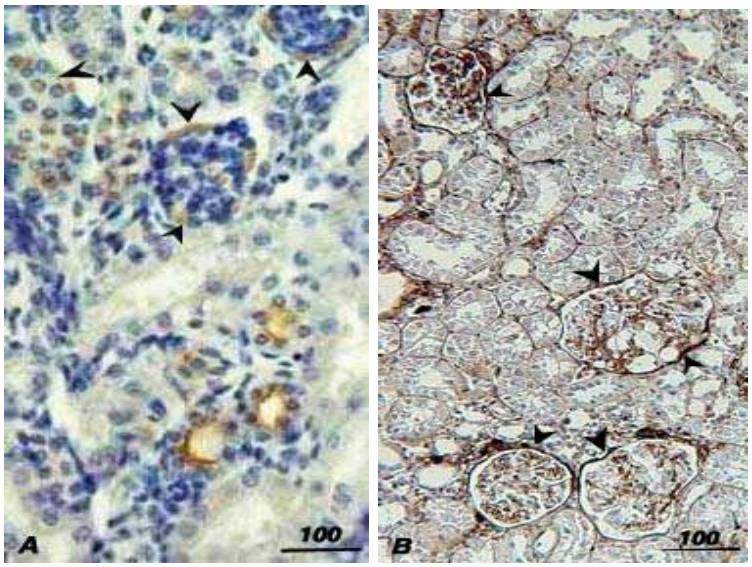

Figure 2. a. Transverse section of glomerulus on day 15 of gestation, The first reaction was observed in basement membrane of glomerulus of cortical regions (arrow heads). b. Sections of glomerulus on 18 day of gestation. In this image it have been showed labeling in both basement membrane of cortical glomerulus (arrow heads).

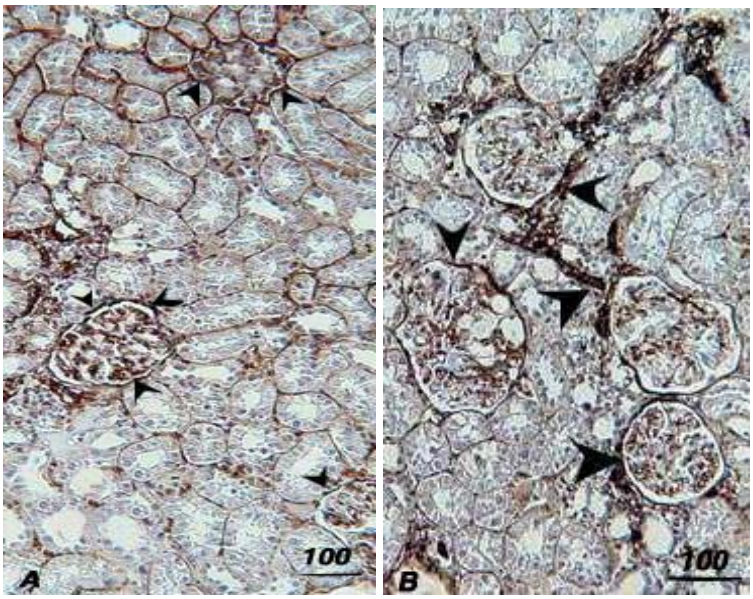

Figure 3. a. Transverse sections through glomerulus on 5th postnatal day, arrowheads refer to strong reaction in glomerular basement membrane. b. Glomerulus on 10th postnatal day, ECM and basement membrane labeling indicate more intensive reaction than previous image.

Table 1. Collagen type IV reaction during kidney glomerular morphogenesis

Embryonic and postnatal days	BMG *	ECM **	PV ***
13th embryonic day	-	-	-
14th = = = =	-	-	+
15th = = = =	+	+	++
18th = = = =	+++	++	++++
5th postnatal day	+++	+++	++++
10th = = = =	++++	++++	++++

Gradation of ranging from negative (zero) to 4 positive in conformity with the severe of reaction from negative, weak, moderate, strong and high strong.

Values represent means ± standard error of the mean (S.E.M), compared to embryonic with postnatal days: * (P 0.002), ** (P 0.005) and *** (P 0.05).

Discussion

Fibrillar proteins especially type IV collagen play different roles in limbs development and tendon junctions (Langmaier et al., 2001). These proteins not only act as conducting system for migration but also they are effective on wound repair, synthesis and turn over components of extra cellular matrix (such as fibronectin, tenasin, collagens, chondroitin sulfate and heparan sulfate), proliferation and cell differentiation (Holster et al., 2007). Interestingly, it has verified that fibrillar proteins such as type IV collagen play important role in repair embryonic wounds without remaining scar (Campos et al., 2008; Figus et al., 2007). Based on our data, it seems that proteins of basement membrane synthesis are required for glomerular epithelium formation. Among them, type IV collagen is the most remarkable component of glomerular basement membrane synthesizes under inductions mechanisms and it reserved in glomerular primordium.

Kidneys are the most important part of urinary tract that its mesenchyme forms tubular epithelium and renal glomerular during different stages of renal morphogenesis (Abrass et al., 2006; Barasch et al., 1999). The embryologic studies show that rudimentary glomerulus appears on 13.5 day of gestation in mouse (Sukhatme et al., 1993). The immunohistochemistry studies of glomerular endothelial formation showed that type IV collagen was first indicated weak reaction on 15 day of gestation. This represents that vessel endothelial formation in glomeruls is required macromolecules of basement membrane especially type IV collagen which plays a crucial rolein this process (Mejelle et al., 2007). Our data indicated that type IV collagen not only increased during final stage of embryonic but also followed on 1-10 of postnatal days.

Based on the previous studies it have been shown that basement membrane density may change by factors such as long-time activity of kidney and increasing age (Gulber et al., 2008). The studies also have revealed that in pathological condition, such as diabetes, renal failure influence on thickness of basement membrane and density of collagen (Funabiki et al., 1998). At this period of time, it have been proved that former protein, is effective on glomerular formation and renal filtration at early stages of kidney development and contributes in an immune response by signal transduction to adjacent tissues and extra cellular matrix that increase thickness of basement membrane and collagen density (Borza et al., 2007). Although this autoimmune response supports glomerular endothelium against chemical factors, it may results in decline of glomerular filtration and renal failure in acute cases. It have been believed that compositions of basement membrane play a structural role in epithelial cells, while have been distinguished basement membrane plays complexity roles in cell behavior such as development, proliferation, morphogenesis, metabolism and pathologic changes (Gullberg et al., 1995). Extra cellular matrix contributes in different cellular activities such as adhesion, migration and signal transduction (Hurle et al. 1989 and Chohen et al., 2007).

In extra cellular matrix, there are 2 groups of fibrillar proteins in extra cellular matrix, structural

proteins such as collagen and elastin. The second one are the proteins that play adhesion role such as laminin and fibronectin (Olson et al., 1991). Among these proteins, collagens are the most abundant component of extra cellular matrix. At least 19 types of collagen have been distinguished so far. Type IV collagen is the most important type of collagen in extra cellular matrix that its crucial role were discussed before. It has been identified that various types of collagen contributes in different morphologic process. The researches have shown that collagens induce endothelial cells to tubular ducts formation (Maeshima et al., 2006). Type II collagen may plays a role in epithelial-mesenchyme interactions that induce to form cytoskeleton (Ishibe et al., 1989). Type VI collagen probably causes cell-matrix interactions and cytoskeleton turn over in fibroblasts that increase cell surface during development (Doane et al., 1992). The studies indicate that extra cellular matrix causes specialized functions in different tissues. For example, the hepatocytes should contact to extra cellular matrix and basement membrane that synthesize specific proteins. Also, numerous rowth factors and hormones transduct signals via binding to extra cellular matrix (Zahang et al., 2007). On the other hand, it is important understanding relation between extra cellular matrix and functional mechanism of growth factors, pathogenesis of diseases and finding new therapeutics (Favor et al., 2007). The increasing type IV collagen represents that glomerular development is dependent to this type of collagen which appeared during mesangeal formation. Collagen detection on 15 days of gestation in glomerular basement membrane and its increase during next days, suggest that gelumerular development is dependent to basement membrane formation.

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