http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 13 (19): 961-965, 2010
ISSN 1028-8880

Pattern of Laminin Expression during Kidney Morphogenesis in Balb/c Mice

[1]Rafighdost Houshang, [2]Nikravesh Mohammad Reza and [2]Jalali Mehdi
[1]Department of Anatomy, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran
[2]Department of Anatomy, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Abstract: Basement membrane of glomerular mesangium (BMG) is one of important components which play a key role to support of the capillary loops in a renal glomerulus and completeness of BMG due to interaction of ureteric bud and metanephric mesenchyme during glomerulogenesis. As laminin contribute in extra cellular matrix and especially in basement membrane, the aim of the present study was to demonstrate the distribution of this molecule so, in this investigation specific antibody against laminin have been used in light microscopy to study development of BMG of fetal and postnatal mouse glomerular mesangium. Female inbred Balb/c mice were selected and were kept under normal condition and finding vaginal plug was assumed as day zero of pregnancy. Two pregnant mice were sacrificed by cervical dislocation in one of gestational days 13-18, respectively and their fetuses were fixed, serially sectioned and by using antibody against laminin in BMG were carried out. The same process was used for kidneys preparation at 15 postnatal days. Present data revealed that laminin showed weak reaction on day 14 of gestation. The amount of laminin increased continuously until next days of fetal life and primary of 10 days postnatal in BMG. After this period, laminin reaction did not show significant change in newborns. These data indicate that laminin appears just during the glomerulogenesis and because of continuity with vasculature which is required for Extra Cellular Matrix (ECM) and glomerular endothelial cell differentiation, laminin, is the one of major structural proteins in BMG.

Key words: Development, extra cellular matrix, basement membrane

INTRODUCTION

Basement membrane is a specialized component of extra cellular matrix which is consists of different compositions such as glycoproteins (Xue *et al*., 1998; Berkholtz *et al*., 2006). In previous investigation, we showed that one of the major basement membrane proteins is type IV collagen (Nikravesh *et al*., 2009; Jalali *et al*., 2009). In addition, there are some other several important protein such as laminin, fibronectin, sulfated and nonsulfated glycosaminoglycans (Berkholtz *et al*., 2006; Mates *et al*., 2004).

Laminin, an extra cellular element is a large, flexible protein composed of three very long polypeptide chains arranged in the shape an asymmetric cross and held together by disulfide bonds. Previous studies showed that laminin plays important roles during development by special regard in extracellular matrix, in addition other studies revealed that this molecule capable to selectively stimulating axonal extension and nurite outgrowth and in Glomerular and Mesenchymal Basement Membrane During Fetal and Postnatal Period of Balb/c Mice (Jalali, 2005). Based on some other findings the BMG not only changes during embryonic period, but also alters at 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) and ECM components turn over continually in developmental organs (Carnegie and Cabac, 1993; Chai *et al*., 2003). Studies have been shown the expression of laminin alpha 1, alpha 5 and beta 2 chains during embryogenesis of the kidney and vasculature (Durbeej *et al*., 1996). Other investigation also demonstrated that an interaction between basement membrane proteins such as collagen type IV and laminins are required for early kidney morphogenesis *in vivo* (Willem *et al*., 2002). Furthermore the data are consistent with a role for laminin-5, acting through its alpha3beta1 and/or alpha6beta4 integrin receptors, in ureteric bud branching during nephrogenesis (Zent *et al*., 2001; Ekblom *et al*., 1990; Klein *et al*., 1988).

In other words, molecules and matrix components are required in cell differentiation and among them, laminin play complex roles in cell behavior such as development, migration, proliferation, morphogenesis and metabolism (Durbeej, 2010). One of the most prominent role of ECM is migration and cell adhesion that laminin molecule serves

Corresponding Author: Jalali Mehdi, Department of Anatomy, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

them (Durbeej, 2010; Urbano *et al*., 2009). Therefore, considering laminin roles in vital organs changes, is necessary to be investigated.

MATERIALS AND METHODS

Twenty four female Balbc/c mice were obtained from animal house of Mashhad University of Medical Sciences in 2009 and selected randomly and finding vaginal plug were assumed as day zero of pregnancy. Two mice per each group were anesthetized by chloroform and were sacrificed by cervical dislocation in one of gestational days 13-18. Their fetuses were collected and were processed for histological studies. The similar processes were carried out for newborns on 1, 5, 10 and 15 of postnatal days. Kidneys were prepared from newborns of 3 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 (containing 1.5% sodium chloride at pH = 7). None-specific antibodies were blocked with 3% Triton X-100 and goat serum for 3 h. For blocking endogenous peroxides activity, sections were treated with 3% H_2O_2- methanol for 1 h and were incubated with the antibody 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 was showed positive reaction according to amount of appearance and the coloring reaction was from light to dark brown. The coloring reaction of laminin was 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 staining laminin was graded by two separate individual according to firth method (Firth and Reade, 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

The results of present study showed that mesenchyme cells are enclosed uretric bud and glomerular primordium and rudimentary tubules observed on day 13 of gestation (Fig. 1). But it was not observed laminin reaction until this period of time. Glomerular development completed on embryonic day 14 and laminin just showed weak reaction in Parenchyma Vessels (PV), (Fig. 2). Laminin showed first reaction on day 15 of gestation in cortical glomerulus (Fig. 3). The intensity of staining

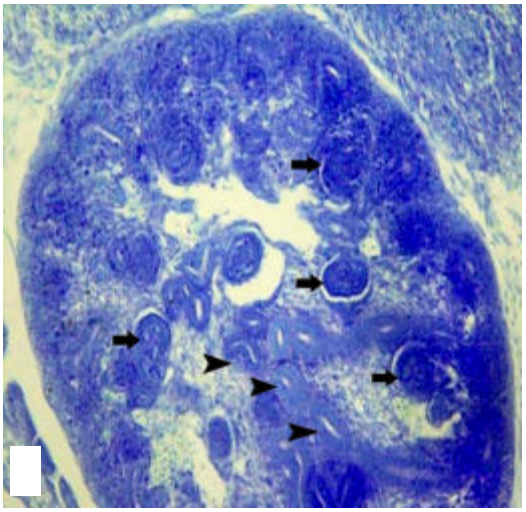

Fig. 1: Transverse section through kidney parenchyme on day 13 of gestation, laminin indicated no reaction in glomerular primordium (arrows) as well as rudimentary tubules (arrowheads)

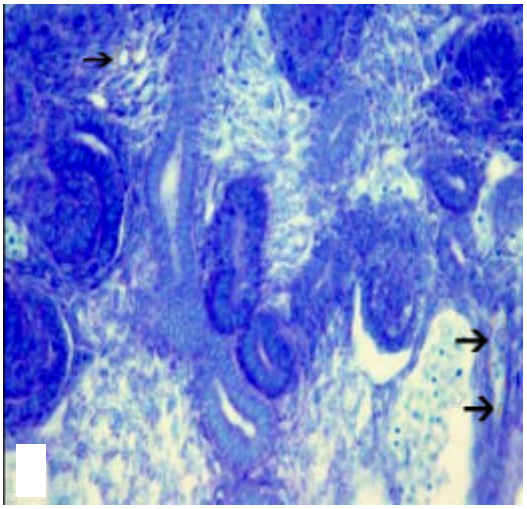

Fig. 2: Other kidney section during glomerular development on day 14 of gestation. Although glomerular structure have been completed, no reaction was observed in any area, but there is a weak laminin reaction (arrows) in parenchyma vessels (Hematoxylin counterstained, Fig. 1, X 100, Fig. 2, X 200)

increased continually in next days and detected on day 18 of gestation in other parts of glomerulus and tubules of kidney (Fig. 4). The results of this stage also showed that sever reaction in tufts of capillaries. The observations of postnatal days indicated that laminin reaction was more intensive on 10 days of postnatal in glomerular and tubular basement membrane (Fig. 5, 6) but did not showed significant changes on after this period (Table 1).

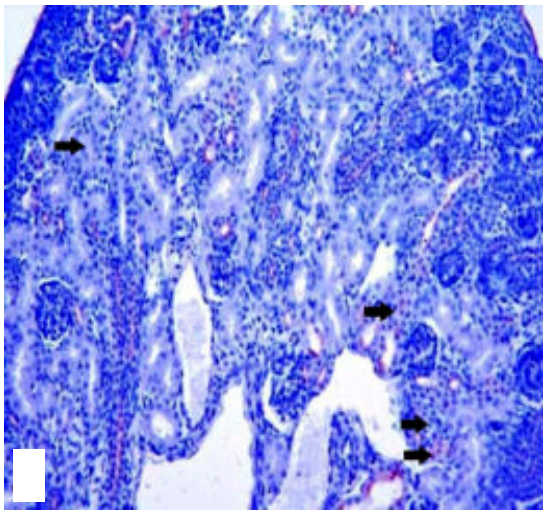

Fig. 3: Transverse section of glomerulus on day 15 of gestation, The first reaction was observed in parenchyma of cortical regions (arrows)

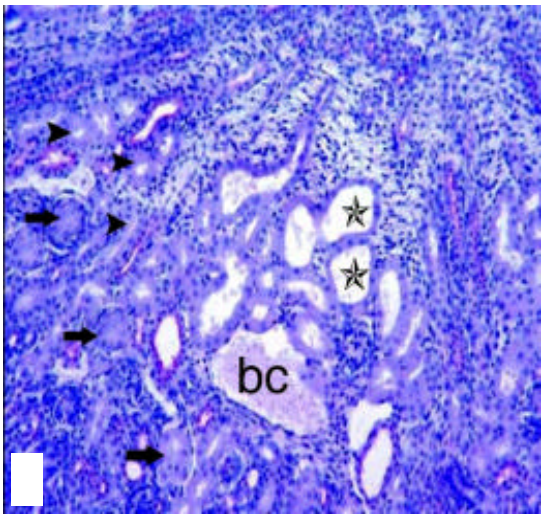

Fig. 4: Sections of glomerulus on 18 day of gestation. In this aspect it have been showed labeling in both cortical glomerulus (arrows) and primary tubules (arrowheads), urethric bud sections (stars), a macro vessel with blood cells (bc) and (X 200)

Table 1: Laminin reaction during kidney glomerular morphogenesis

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

This gradation was scored 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±SE of the mean (SEM), compared to embryonic with postnatal days: *p≤0.002, **p≤0.005 and ***p≤0.05

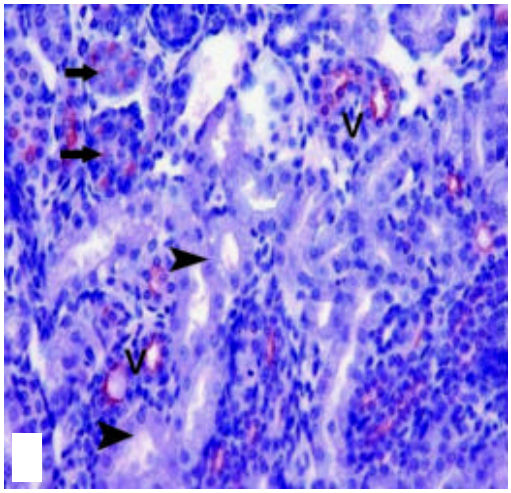

Fig. 5: Transverse sections through glomerulus on 5th postnatal day, arrows refer to strong reaction in glomerular mesangium, tubules (arrowheads) and micro vessels (V)

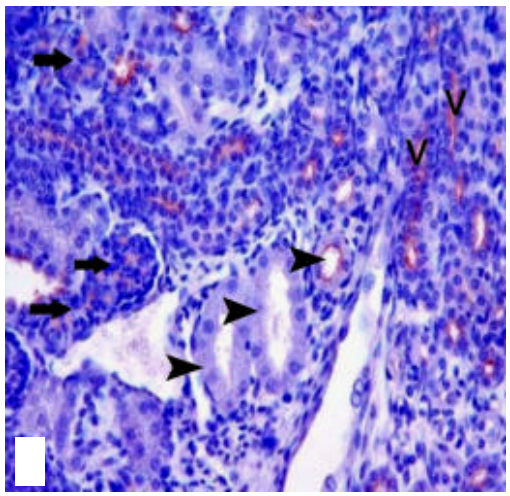

Fig. 6: Glomerulus on 10th postnatal day, that laminin glomerulus reaction in (arrows), tubules (arrowheads) and parenchyma vessels (V) labeling indicate more intensive reaction than previous days

DISCUSSION

The kidney is one of the main parts of urinary system that its mesenchyme forms tubular epithelium and renal glomerular during different stages of development (Abrass *et al*., 2006; Barasch *et al*., 1999). The embryologic studies show that rudimentary glomerulus appear on 12.5-13 days of gestation in mouse (Ekblom *et al*., 1980). In connection with kidney development, there are 2 groups of fibrillar proteins in extra cellular matrix: structural proteins such as collagen and elastin and proteins which play adhesion role such as laminin and fibronectin (Olson *et al*., 1991). It has been suggested that the vessel endothelial formation in glomerulus is required to basement membrane support that its proteins especially laminin plays a crucial role (Urbano *et al*., 2009). The present data show that, laminin indicated weak reaction on 14 day of gestation in glomerular endothelial formations. The first evidence of laminin was appeared around the inductor tissue in the undifferentiated mesenchyme. These results also showed that laminin not only increased during final stage of embryonic but also followed on 1-10 of postnatal days. Of course, it does not mean that its density does not change by factors such as long-time activity of kidney and increasing age (Karttunen *et al*., 1986). These data are apparently confirmed by other investigation that suggest laminin was regularly found distributed as spots around the tips of the ureter at places known to aggregate first (Ekblom *et al*., 1980). The studies have been shown that in pathological condition, such as diabetes and renal failure may influence on thickness of basement membrane and change of Laminin (Pugliese *et al*., 1997). This protein effects on glomerular formation and renal filtration at early stages of kidney development (Abrahamson, 2009). It also contributes in an immune response by signal transduction to adjacent tissues and extra cellular matrix that increase thickness of basement membrane (Abrass *et al*., 1995). Of course, this autoimmune response supports glomerular endothelium against chemical factors, which results in decrease of glomerular filtration and renal failure in acute cases (Yasuda *et al*., 1996). It have been also 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 and Ekblom, 1995). Glomerular extra cellular matrix contributes in different cellular activities such as adhesion, migration that fibrillar proteins such as laminins, serve them (Hamill *et al*., 2009). Interestingly, it has verified that fibrillar proteins such as laminin play unique and synergistic role with other proteins during development (Peterson and Henry, 2010). Also the Laminins are key components of basement membranes and are thought to be essential for initiation of basement membrane assembly (Anderson *et al*., 2009). At this rate, laminin is one of the most remarkable component of glomerular basement membrane synthesizes under inductions mechanisms and it reserved in glomerular primordium and increasing of this molecule represents that glomerular development is dependent to laminin that appeared during mesangeal formations. In the other hand, laminn detected on 14 day of gestation in glomerular basement membrane and increased in next days that suggests gelumerular development is dependent to basement membrane formation. After birth, when glomerulus developed laminin density did not show change in newborns. This data indicate that the expression of laminin in the developing kidney is a necessary for shaping of basement membranes and glomerulogenesis.

ACKNOWLEDGMENT

The authors wish to thank Mrs. Motejadded for her excellent technical assistance.

REFERENCES

Abrahamson, D.R., 2009. Development of kidney glomerular endothelial cells and their role in basement membrane assembly. Organogenesis, 5: 275-287.

Abrass, C.K., M.J. Adcox and G.J. Raugi, 1995. Aging-associated changes in renal extracellular matrix. Am. J. Pathol., 146: 742-752.

Abrass, C.K., A.K. Berfield, M.C. Ryan, W.G. Carter and K.M. Hansen, 2006. Abnormal development of glomerular endothelial and mesangial cells in mice with targeted disruption of the lama3 gene. Kidney Int., 70: 1062-1071.

Anderson, C., S. Thorsteinsdóttir and A.G. Borycki, 2009. Sonic hedgehog-dependent synthesis of laminin alpha1 controls basement membrane assembly in the myotome. Development, 136: 3495-3504.

Barasch, J., J. Yang, C.B. Ware, T. Taga and K. Yoshida *et al*., 1999. Mesenchymal to epithelial conversion in rat metanephros is induced by LIF. Cell, 99: 377-386.

Berkholtz, C.B., B.E. Lai, T.K. Woodruff and L.D. Shea, 2006. Distribution of extracellular matrix proteins type I collagen, type IV collagen, fibronectin and laminin in mouse folliculogenesis. Histochem. Cell Biol., 126: 583-592.

Carnegie, J.A. and O. Cabac, 1993. Extracellular matrix composition and resilience: Two parameters that influence the *in vitro* migration and morphology of rat inner cell mass-derived cell. Biol. Reprod., 48: 287-299.

Chai, Q., S. Krag, J.H. Miner, J.R. Nyengaard, S. Chai and L. Wogensen, 2003. TGF-beta1 induces aberrant laminin chain and collagen type IV isotype expression in the glomerular basement membrane. Nephron Exp. Nephrol., 94: 123-136.

Cosgrove, D., D.T. Meehan, D. Delimont, A. Pozzi and X. Chen *et al*., 2008. Integrin alpha1beta1 regulates matrix metalloproteinases via P38 mitogen-activated protein kinase in mesangial cells: Implications for Alport syndrome. Am. J. Pathol., 172: 761-773.

Durbeej, M., L. Fecker, T. Hjalt, H.Y. Zhang and K. Salmivirta *et al*., 1996. Expression of laminin alpha 1, alpha 5 and beta 2 chains during embryogenesis of the kidney and vasculature. Matrix Biol., 15: 397-413.

Durbeej, M., 2010. Laminins. Cell Tissue Res., 339: 259-268.

Ekblom, P., K. Alitalo, A. Vaheri, R. Timpl and L. Saxen, 1980. Induction of a basement membrane glycoprotein in embryonic kidney: Possible role of laminin in morphogenesis. Dev. Biol., 77: 485-489.

Ekblom, M., G. Klein, G. Mugrauer, L. Fecker, R. Deutzmann, R. Timpl and P. Ekblom, 1990. Transient and locally restricted expression of laminin A chain mRNA by developing epithelial cells during kidney organogenesis. Cell, 60: 337-346.

Firth, N.A. and P.C. Reade, 1996. The prognosis of oral mucosal squamous cell carcinomas: A comparison of clinical and histopathological grading and of laminin and type IV collagen staining. Aust. Dental J., 41: 83-86.

Gullberg, D. and P. Ekblom, 1995. Extracellular matrix and its receptors during development. Int. J. Dev. Biol., 39: 845-854.

Hamill, K.J., K. Kligys, S.B. Hopkinson and J.C. Jones, 2009. Laminin deposition in the extracellular matrix: A complex picture emerges. J. Cell Sci., 122: 4409-4417.

Jalali, M., 2005. Folic acid reduced focal cortical dysplasia of the cerebellum by induction of laminin expression in C57BL/6J mice. Iran. J. Basic Med. Sci., 8: 60-66.

Jalali, M., M.R. Nikravesh, A.A. Moeen, M.H. Karimfar, S. Saidinejat, S. Mohammadi and H. Rafighdoost, 2009. Inductive role of type IV Collagen in nephrogenesis. Urol. J., 6: 289-294.

Karttunen, T., J. Risteli, H. Autio-Harmainen and L. Risteli, 1986. Effect of age and diabetes on type IV collagen and laminin in human kidney cortex. Kidney Int., 30: 586-591.

Klein, G., M. Langegger, R. Timpl and P. Ekblom, 1988. Role of laminin: A chain in the development of epithelial cell polarity. Cell, 55: 331-341.

Mates, L., C. Nicolae, M. Morgelin, F. Deak, I. Kiss and A. Aszodi, 2004. Mice lacking the extracellular matrix adaptor protein matrilin-2 develop without obvious abnormalities. Matrix Biol., 23: 195-204.

Nikravesh, M.R., M. Jalali, A.A. Moeen, M.H. Karimfar, S.S. Nejat, S. Mohammadi and H. Rafighdoost, 2009. The role of type IV Collagen in developing eye lens in mouse fetuses. Iran. J. Basic Med. Sci., 12: 158-162.

Olson, A.D., T. Pysher and R.S. Bienkowski, 1991. Organization of intestinal epithelial cells into multi cellular structure requires laminin and functional actin microfilament. Exp. Cell Res., 192: 543-549.

Peterson, M.T. and C.A. Henry, 2010. Hedgehog signaling and laminin play unique and synergistic roles in muscle development. Dev. Dyn., 239: 905-913.

Poschl, E., U. Schlptzer-Schrehardt, B. Brachvogel, K. Saito, Y. Ninomiya and U. Mayer, 2004. Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development. Development, 131: 1619-1628.

Pugliese, G., F. Pricci, P. Mene, G. Romeo and I. Nofroni *et al*., 1997. High glucose level unmasks a genetic predisposition to enhanced extracellular matrix production in mesangial cells from the Milan normotensive strain. J. Am. Soc. Nephrol., 8: 406-414.

Urbano, J.M., C.N. Torgler, C. Molnar, U. Tepass and A. Lopez-Varea, 2009. Drosophila laminins act as key regulators of basement membrane assembly and morphogenesis. Development, 136: 4165-4176.

Willem, M., N. Miosge, W. Halfter, N. Smyth, I. Jannetti, E. Burghart, R. Timpl and U. Mayer, 2002. Specific ablation of the nitrogen-binding site in the laminin gamma1 chain interferes with kidney and lung development. Development, 29: 2711-2722.

Xue, Y., J. Li, M.A. Latijnhouwers, F. Smedts and R. Umbas, 1998. Expression of periglandular tenascin-C and basement membrane laminin in normal prostate, benign prostatic hyperplasia and prostate carcinoma. Br. J. Urol., 81: 844-851.

Yasuda, T., S. Kondo, T. Homma and R.C. Harris, 1996. Regulation of extracellular matrix by mechanical stress in rat glomerular mesangial cells. J. Clin. Invest., 98: 1991-2000.

Zent, R., K.T. Bush, M.L. Pohl, V. Quaranta and N. Koshikawa *et al*., 2001. Involvement of laminin binding integrins and laminin-5 in branching morphogenesis of the ureteric bud during kidney development. Dev. Biol., 238: 289-302.