Original Article

Evalauation of Laminin Expression during Mouse Lens Development

Houshang Rafighdoost, Ph.D.[1], Mehdi Jalali, Ph.D.[2], Mohammad Reza Nikravesh, Ph.D.[2*]

1.Department of Anatomy, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran
2.Department of Anatomy and Cell Biology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
***Corresponding author,** E-mail address: Doctornikravesh@yahoo.com*

Received: December 2011 Accepted: March 2012

Professor Mohammad Reza Nikravesh is a Professor of Anatomy in the Department of Anatomy and Cell Biology, Mashhad University of Medical Sciences, Mashhad, Iran. Dr. Nikravesh has over thirty years of experience as an educator of anatomical sciences, supervisor of research, and researcher in basic medical sciences. His research focuses on collagen type IV, laminin and fibronectin, key proteins with important functions in the basement membrane and extracellular matrix.

Abstract

Introduction: Among the components of the extracellular matrix (ECM) and basement membrane (BM), laminitis heterotrimeric glycoprotein (laminin) and collagen type IV are the most important. In a previous study we have examined the role of collagen type IV in the developing lens capsule. The present study aims to determine the appearance and distribution of laminin in the BM and ECM of lenses during visual system morphogenesis.
Materials and Methods: Pregnant Balb/C mice were randomly selected and maintained under normal conditions. The presence of a vaginal plug was assumed as day zero of pregnancy. From embryonic days 11 to 20, pregnant animals were sacrificed and their fetuses were collected for histotechnical prossessing.
Results: Our data revealed that laminin appeared during the early stage of gestation (day 12) in the BM of the anterior epithelial lens cells. The amount of laminin gradually increased in the ECM and posterior lens capsule epithelial cells until days 14-18. After this period, a strongly positive laminin reaction was not observed in any part of the lens structure.
Conclusion: These findings establish the importance of the laminin molecule in the developing optic cup (OC) and lens differentiation. It could be assumed that any changes in the presence of laminin during the critical period of eye development may result in visual system defects such as cataracts or congenital eye abnormalities.
Keywords: Laminin, Mice, Lenses, Growth and development

To cite this paper: Rafighdoost H, Jalali M, Nikravesh MR. Evalauation of laminin expression during mouse lens development. Anat Sci J 2013; 10(1): 1-6.

Introduction

Laminitis heterotrimeric glycoprotein (laminin) is one of the major components of the basement membrane (BM) and extracellular matrix (ECM) in most animal tissues [1]. Laminin is one of the larger proteins that play an important role in eye lens and optic cup (OC) morphogenesis [2,3]. Our recent investigations have shown that other ECM components such as collagen type IV are also important during lens morphogenesis [4,5]. The lens is a transparent, flexible component of the optic system which develops along with the other parts of the eyeball [6,7].

Researches have shown that defects in laminin expression can cause congenital defects of the nerves and OC in animal models [8,9]. The absence of laminin subunits (alpha, beta, and gamma precursors) causes not only lens deformation but also fragility and rupture of the capsule [10]. A mutation in any of the laminin subunits can result in defects in laminin synthesis and bring about a change in components of the eye's structures [5].

Laminin synthesis is one of the most important processes related to the development and shape of the eye lens during morphogenesis [11]. Based on different studies, various types of proteins have been shown to comprise the structure of the lens capsule such as collagen type IV, entactin/nidogen, heparin sulfate and some glycoproteins [5]. Although, we should not ignore the structural role of lens proteins. Laminin is one of the most abundant molecules in the eyeball and plays a critical role in lens development [11]. In the present study we investigate the appearance and distribution of laminin in the BM and ECM of mice lenses during visual system morphogenesis.

Materials and Methods

20 pregnant balb/c mice, were obtained from the animal house of Mashhad University of Medical Sciences. Two pregnant mice at rather embryonic days E_{11} to E_{20} were anesthetized and perfused transcardially with formaldehyde (10%). The heads of the fetuses were removed and post-fixed for 24 h at room temperature in the same fixative. The fetal heads were routinely processed, embedded in paraffin, then serially sectioned into 8 μm sagittal sections. Sections were either stained with cresyl violet or incubated with monoclonal antibody against laminin α5.

All study procedures were approved by the Medical Ethics Committee Mashhad University of Medical Sciences, Mashhad, Iran.

We used the avidin-biotin-peroxidase immuno-histochemical procedure for analysis of the serial sections. These sections were deparaffinized, rehydrated and washed twice for 5 min in 0.05 Tris buffer that contained 1.5% NaCl (pH 7.4). For blocking any nonspecific antibody, sections were preincubated in 0.3% Triton X-100 in Tris buffer NaCl (TB-NaCl) followed by 5% goat serum for 2-3 h. Then, sections were allowed to react for 12-24 h at 4°C with primary antibody (anti-laminin 2413, Dako Co., USA) diluted 1:250 in TB-NaCl with 0.3% Triton X-100 and 2% serum. Tissues were washed three times with TB-NaCl, for 10 min each time and then incubated for 2 h in biotinylated goat anti-rabbit IgG (1:400 in TB-NaCl). After three additional rinses, each for 1 h, endogenous peroxidase activity was blocked by incubation in 0.03% H_2O_2 in methanol for 30 min. Tissues were then incubated for 2 h in a 1:100 avidin-biotinylated horseradish peroxidase complex, washed three times (30 min each wash) in TB-NaCl and allowed to react with a 0.03% solution of 3,3-diaminobenzidine tetra- hydrochloride that contained 0.03% H_2O_2 for 10-15 min. Tissues were washed and lightly counterstained with

hematoxylin. Subsequently, tissues were washed, air-dried, dehydrated, and then mounted in PBS glycerol. Photographs were taken by a BX52 Olympus light microscope was appointed to camera. wichs.

The laminin reaction in the lens components for each of the embryonic days was evaluated according to the method by Firth and Read. Grading was scored according to the severity of the reaction, as follows: negative (-), weak (+), moderate (++), strong (+++), and highly strong (++++). The intensity of staining was graded by two individuals, separately, according to the above method [12,13].

Statistical analysis

Data were analyzed by SPSS software (Version 19), using the Kruskal-Wallis and Mann-Whitney tests. $P<0.05$ were considered statistically significant.

Results

Our findings showed that the primary stage of OC development on day 10 of gestation (Fig. 1a) and the primary lens (PL) was primary cellular mass in the anterior pole of the OC (Fig. 1b). On day 11 the PL cellular mass acquired a lenticular shape (L). In this stage, although the epithelium of the lens was formed, however there was no laminin reaction in the BM (Fig. 1c). On day 12, the lens structure changed to a cortical region. In addition we observed a weak laminin reaction in the nuclear region of the lens (Fig. 1d). Based on our findings, laminin clearly appeared by embryonic day 14 (Fig. 1e) in the lens matrix (LM) and BM of the epithelial cells (arrows). There was a population of primordial cells (head arrows) in the LM on day 15, however a cellular degeneration process occurred with replacement by laminin fibers in the lens nucleus, particularly in the lens cortex on day 16 (Fig. 1g and 1h), respectively. Over days 17 and 18 the BM of epithelial cells (Figure 1i) and posterior lens capsule (Fig. 1j) showed very strong reactions with antibody against laminin (Table 1), however there was a conspicuous population of the remnants of primordial cells in these areas.

Table 1. Laminin reaction in the lens components during mouse embryonic development.

Embryonic days	Basement membrane (BM)	Extracellular matrix (ECM)		
	Anterior epithelial cells	Nucleus	Cortex	Posterior capsule
10	-	-	-	-
11	-	-	-	-
12	+	+	-	-
13	++	+	+	+
14	+++	++	+++	++
15	+++	++	++++	++
16	++++	++	++++	+++
17	++++	++	++++	+++
18	++++	+++	++++	++++

Scored according to the extent of the laminin reaction,as follows: negative (-), weak (+), moderate (++), strong (+++), and very strong (++++)

Values that are (-), (+) and (++) are not significant

+++ values: $p<0.005$

++++ values: $p<0.0005$

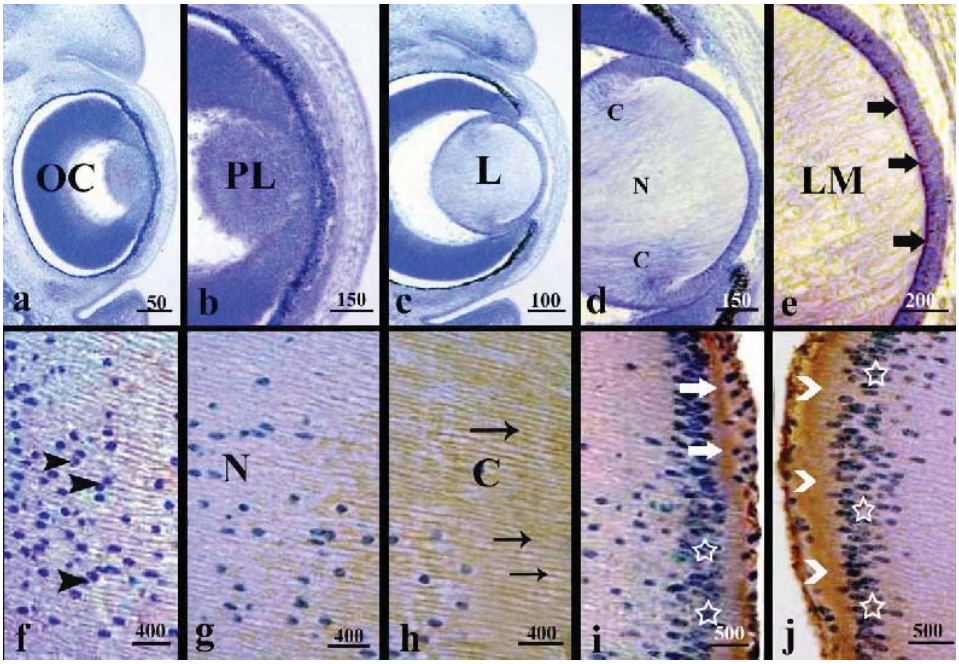

Figure 1. Images a-j showing sections through the eyeball through days 10-18 of gestation respectively which are incubated with monoclonal antibody against laminin. a and b: on day 10th of gestation, optic cup (OP) and primary lens (PL). c: on day 11th of gestation, the lens matrix (L) d: day 12, weakly reaction in basement membrane of anterior epithelium (arrows) and nuclear region (c: cortex and n: nucleus) e: section through lens on day 14 of gestation. Anterior epithelium basement membrane (arrows) and lens matrix (LM) with remarkable reaction. f: middle part of lens on day 15 of gestation with higher magnification which clearly showed laminin reaction in lens nucleus and many primordial cells reminded (head arrows). On day 16th of gestation (g and h), g: section through lens nucleus (n) with weak laminin reaction and h: cortical lens region (c) with severe laminin reaction (arrows). On day 18th of gestation (i and j), i: through lens epithelium basement membrane (arrows) j: posterior capsule (bifurcated arrows) with severe laminin reaction and remains of primordial cells (asterisks) in both figures (scale bar =µm, Hematoxylin counterstained).

Discussion

Our previous studies have shown that BM and ECM molecules play an important role in tissue development and promotion of cell adhesion, migration, growth and differentiation [5,14,15]. Among these molecules, laminin is a large glycoprotein which is one of the major components of BM.

According to the results of our immuno-histochemical studies, there was no reaction of laminin until gestational day 12. In another investigation, the initial laminin expression in the Cat Fraser mouse was detected in the lens capsule as early as embryonic day 10 [16]. The above mentioned investigation has also shown that, at this stage, other important BM proteins such as collagen type IV had a similar distribution as laminin and fibronectin. In our previous immunohistochemical studies there was no collagen type IV reaction until day 12 of gestation in the lens structure [5].

The appearance of the first signals of laminin expression in the BM of the anterior capsule possibly represented the important role of this molecule in lens development. Investigations have shown that the anterior capsule consists of specialized BM to which epithelial cells bind [17]. As the structural compositions of the lens become completed, the specific role of laminin is distinguished in this part of the visual system. Since the marginal zone of the capsule binds to

connective bundles (zonules) which connects the capsule to the ciliary body by using stable, thin fibers the strength force diffuses to the lens surface [18]. Thus compositions such as collagen, fibronectin and laminin are required to provide stability and flexibility [19,20]. Subunits of these molecules can bind to cell-surface and intra-cellular receptors, affecting compositions of the LM [21].

Analysis of the emergence of laminin at later gestational days, especially day 14 of gestation, showed that its density significantly increased from the anterior to the LM. This density peaked on day 18 of gestation when the capsule as well as structural composition of the lens became full of laminin. However laminin was poorly reactive in the nuclear region of the lens. Here, the distribution pattern did not show a distinguished change over the following days.

Therefore, in order to have a better understanding of the formation, development and role of laminin in accommodation, it is necessary to obtain information on the appearance and distribution of private molecules such as laminin, fibronectin, and collagen type IV and their alterations [5,22]. As our data indicated, the appearance of laminin at gestational day 12 and its increase until embryonic day 18 might be an index of developmental changes in the optic lens. At the completion of lens development, laminin synthesis ceases. During these stages, the cell mass, which is known as the lens precursor, gradually disappears and is replaced with macromolecules such as collagen IV, laminin and fibronectin [23]. Similar immunohistochemical studies on the abnormal appearance of laminin in the pre- and postnatal stages of transgenic mice have shown that laminin forms on day 10 of gestation where it expands until after birth [6,7]. These findings establish the importance of laminin during the critical period of lens development. This study has shown the presence of elevated levels of laminin at the BM of anterior epithelial cells, the posterior capsule, and cortical region during early lens development during growth of the anterior epithelial cell BM and posterior capsule.

Acknowledgment

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

References

1. Ekblom P, Lonai P, Talts JF. Expression and biological role of laminin-1. Matrix Biol 2003; 22: 35-47.

2. Hallmann R, Horn N, Selg M, Wendler O, Pausch F, Sorokin LM. Expression and function of laminins in the embryonic and mature vasculature. Physiol Rev 2005; 85: 979-1000.

3.Parmigiani CM, Mcavoy JW. The roles of laminin and fibronectin in the development of the lens capsule. Curr Eye Res 1991; 10**:** 501-11.

4. Saika S, Miyamoto T, Ishida I, Barbour WK, Ohnishi Y, Ooshima A. Accumulation of thrombospondin-1 in post-operative capsular fibrosis and its down-regulation in lens cells during lens fiber formation. Exp Eye Res 2004; 79**:**147-56.

5. Nikravesh MR, Jalali M, Moein AA, Karimfar MH, Saeidinezhat Sh. The Role of Type IV Collagen in Developing Lens in Mouse Fetuses. Iran J Basic Med Sci 2009; 12: 158-62.

6. Parmigiani C, Mcavoy J. Localisation of laminin and fibronectin during rat lens morphogenesis. Differentiation 1984; 28**:** 53-61.

7. Parmigiani CM, Mcavoy JW. The roles of laminin and fibronectin in the development of the lens capsule. Curr Eye Res 1991; 10**:** 501-11.

8. Zwaan J, Webster EH Jr. Histochemical analysis of extracellular matrix material during embryonic mouse lens morphogenesis in an aphakic strain of mice. Dev Biol 1984; 104: 380-89**.**

9. Rossi M, Morita H, Sormunen R, Airenne S, Kreivi M, Wang L, et al. Heparan sulfate chains of perlecan are indispensable in the lens capsule but not in the kidney. EMBO J 2003; 22: 236-45.

10. Dong LJ, Chung AE. The expression of the genes for entactin, laminin A, laminin B1 and laminin B2 in

murine lens morphogenesis and eye development. Differentiation 1991; 48: 157-72.

11. Wederell ED, Brown H, O'connor M, Chamberlain, CG, Mcavoy JW, De Iongh RU. Laminin-binding integrins in rat lens morphogenesis and their regulation during fibre differentiation. Exp Eye Res 2005; 81(3):326-39.

12. Rafighdoust H, Nikravesh MR, Jalali M.. Pattern of laminin expression during kidney morphogenesis in Balb/c mice. Pakistan J Biol Sci 2010, 13(19): 961-65.

13. Firth NA, Reade PC. The prognosis of oral mucosal squamous cell carcinomas: a comparison of clinical and histopathological grading and of laminin and type IV collagen staining. Aust Dent J 1996; 41: 83-6.

14. Nikravesh MR, Jalali M, Moein AA, Karimfar MH, Mohammadi Sh, Rafighdoost H. Study of Basement Membrane Type IV Collagen Appearance in the Brain Choroids Plexus of Mouse Fetuses. Scientific J Hamadan Univ Med Sci Health Serv 2009; 16: 5-9.

15. Jalali M, Nikravesh MR, Moeen AA, Karimfar MH, Saidinejat S, Mohammadi S, et al. Inductive role of collagen type IV during nephrogenesis in mice. Urol J 2009; 6: 289-94.

16. Haloui Z, Jeanny JC, Jonet L, Courtois Y, Laurent M. Immunochemical analysis of extracellular matrix during embryonic lens development of the Cat Fraser mouse. Exp Eye Res 1988; 46**:** 463-74.

17. Johnson MC, Beebe DC. Growth, synthesis and regional specialization of the embryonic chicken lens capsule. Exp Eye Res 1984; 38: 579-92.

18. Pazour GJ, Bloodgood RA. Targeting proteins to the ciliary membrane. Curr Top Dev Biol 2008; 85: 115-49.

19. Inoue S, Leblond CP. Three-dimensional network of cords: the main component of basement membranes. Am J Anat 1988; 181: 341-58.

20. De Jong-Hesse Y, Kampmeier J, Lang GK, Lang GE. Effect of extracellular matrix on proliferation and differentiation of porcine lens epithelial cells. Graefes Arch Clin Exp Ophthalmol 2005; 243: 695-700.

21. Greiling TM, Houck SA, Clark JI. The zebrafish lens proteome during development and aging. Mol Vis 2009; 15**:** 2313-25.

22. Olivero DK, Furcht LT. Type IV collagen, laminin, and fibronectin promote the adhesion and migration of rabbit lens epithelial cells in vitro. Invest Ophthalmol Vis Sci 1993; 34: 2825-34.

23. Taliana L, Evans MD, Ang S, Mcavoy JW.. Vitronectin is present in epithelial cells of the intact lens and promotes epithelial mesenchymal transition in lens epithelial explants. Mol Vis 2006; 12: 1233-42.