Eur J Anat, 4 (1): 1-5 (2000)

Tenascin expression during the postnatal development of mesangium in murine kidneys

José L. Ojeda

Department of Anatomy and Cell Biology. University of Cantabria, Spain

SUMMARY

It is now widely acknowledged that extracellular matrix (ECM) activates predetermined developmental programs to induce the differentiation of epithelial cells. Tenascin (TN) is an ECM glycoprotein that plays a role in mediating cell-matrix adhesion. In mature mammalian kidneys TN is an important component of mesangial matrix. However, its time course and spatial patterns of expression during renal corpuscle morphogenesis have not been established. This study used immunohistochemistry to examine TN expression during the postnatal development of mesangium in the renal corpuscle of murine kidneys. Four different stages of renal corpuscle development were considered: S-shaped body, developing loop capillary, maturing renal corpuscle, and mature renal corpuscle. In the developing capillary stage, TN expression began in the mesangium, and in this stage TN immunoreactivity was also seen in the hilus of the renal corpuscle. High levels of TN expression were observed in both the mesangium and the ECM of the afferent and efferent arterioles in the maturing and mature renal corpuscle stages.

The present results show that the first appearance of mesangial cells and the beginning of TN reactivity in the mesangial ECM are concomitant processes, which suggests that TN reactivity may constitute a useful marker to study the development and maturation of mesangium.

Key Words: Extracellular matrix - Renal corpuscle - Immunohistochemistry - Mesangium - Tenascin

INTRODUCTION

The extracellular matrix (ECM) is a complex assembly of molecules that interact with one another as well as with cells, by means of integrin receptors, to effect a wide range of cellular functions. The ECM plays a key role not only in normal organogenesis (for review see: Hay, 1991) but also in the pathogenesis of several diseases (for review see: Martinez Hernandez and Amenta, 1983).

The tenascins are a family of three ECM glycoproteins namely tenascin-C (TN), Tenascin-R and tenascin-X (Ericson, 1993). TN was the first tenascin to be discovered and there is a wealth of information on its tissue distribution (for review see: Chiquet, 1992). The functions of TN are not well known. Gene knockout experiments in transgenic mice have shown that mice develop normally without TN (Saga et al., 1992). However, data on its tissue distribution and from culture experiments suggest that TN is an adhesion-modulating protein (Chiquet-Ehrismann, 1995).

During nephrogenesis, TN is expressed in the ECM of the condensate mesenchyme surrounding the developing kidney tubule epithelia (Aufderheide et al., 1987; Truong et al., 1996). In mature kidneys, TN is an important component of normal mesangial matrix (Koukoulis et al., 1991; Truong et al., 1996). Although the functional role of mesangial TN has not been determined, the data obtained from experimental glomerulonephritis suggest that this ECM protein plays an important role in mesangial cell proliferation and function (Nakao et al., 1998). The time course and spatial patterns of TN mesangial expression during the morphogenesis of renal corpuscle have not been established. However,

Submitted: February 3, 2000
Accepted: April 14, 2000

Correspondence to:
Profesor José L. Ojeda. Departamento de Anatomía y Biología Celular. Facultal de Medicina. Cardenal Herrera Oria, s/n. 39011 Santander, Spain
E-mail: ojedajl@medi.unican.es

this information may be relevant for a better understanding of the correlation between mesangial cell development and differentiation and TN expression.

The neonatal mouse kidney - and the kidneys of other mammals with a high degree of immaturity at birth - constitutes an excellent model for the investigation of renal development, differentiation, and maturation. A single, well-oriented corticomedullary tissue section allows observation of all stages of nephron morphogenesis and maturation.

In this study, I used immunohistochemistry staining to investigate TN expression during the postnatal development of mesangium in the renal corpuscle of the mouse kidney in an attempt to achieve a better understanding of the development, differentiation, and maturation of mesangium.

Material and Methods

Normal C57BL/6J mice (n=12) between 1 and 25 postnatal days were used in this study.

For TN immunolocalization, the kidneys from anesthetized mice were fixed by intravascular perfusion through the heart with ethanol-glacial acetic (99:1). After perfusion, the kidneys were removed and sliced with a razor blade. Kidney slices were immersed in fresh cold fixative for an additional 30 min, dehydrated in cold ethanol, transferred to cold xylene, and embedded in diethylene glycol distearate (DGD) (Polysciences, Warrington, Pa), as previously described (Ojeda et al., 1989). Briefly, the kidney fragments were transferred from the xylene to a 2:1 mixture of xylene/DGD, and then to a 1:2 mixture (10 min each), followed by two changes of 100% DGD (15 min each). During the embedding procedure, the DGD was maintained at 601/4C to keep it molten. After infiltration, the kidney fragments were transferred to a flat silicone embedding mould filled with freshly melted DGD. After solidification at room temperature, the DGD blocks were stored at 4°C until sectioning.

Sections were cut at 2µm by means of a Jeol Jum-7 ultramicrotome with glass knives and a water bath. The sections were removed from the water with a glass rod and transferred to a water drop on a clean slide. The slides were placed on a warm plate (40°C) until dry and stored at 4°C overnight.

TN was localized in the sections using an extravidin-biotin-peroxidase kit (Sigma). Dewaxed sections were first treated with 3% hydrogen peroxide to block endogenous peroxidase and then incubated with the primary antibody (dilution 1:50) for 6 h at room temperature. Monoclonal anti-mouse TN (Sigma) was used in this study. Omission of the primary and/or secondary antisera from the immunocytochemical procedure eliminated all positive staining. After incubation, sections were processed according to the detection kit protocol. The sections were lightly counterstained with Mayer's hematoxylin (Sigma).

The stained sections were sealed on the slides with Crystal/Mount (Biomeda, Foster City, CA).

Results

General description of renal corpuscle development

The metanephros or definitive kidney is generally accepted to be derived from two sources: all epithelial cell types of the nephron from the metanephrogenic mesenchyme and the collecting ducts from the ureteric bud (Du Bois, 1969). A third important component of the kidney is the vasculature. In the renal corpuscle, this consists of a complex of capillary loops and associated mesangial cells.

Renal corpuscle development is a continuous process which for convenience has been divided into stages. For my purposes it has proved convenient to recognize the following four stages (Reeves et al., 1978; Ojeda et al., 1993): S-shaped body, developing capillary, maturing renal corpuscle, and mature renal corpuscle.

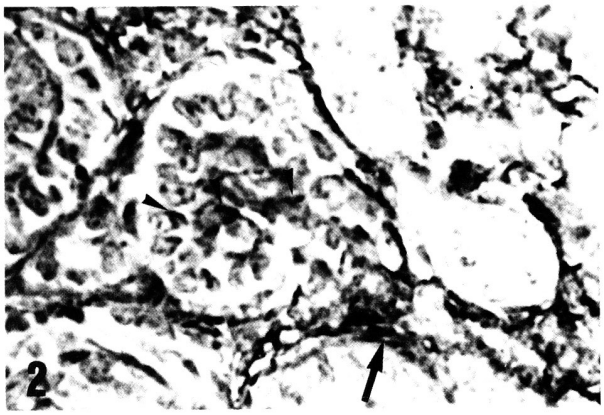
In the kidney of a 1- to 6-day-old mouse there is a gradient in the degree of development so that the more mature renal corpuscles are located toward the corticomedullary junction, and immature renal corpuscles are located towards the renal capsule. Only mature renal corpuscles can been seen in the 10-day-old mouse kidney.

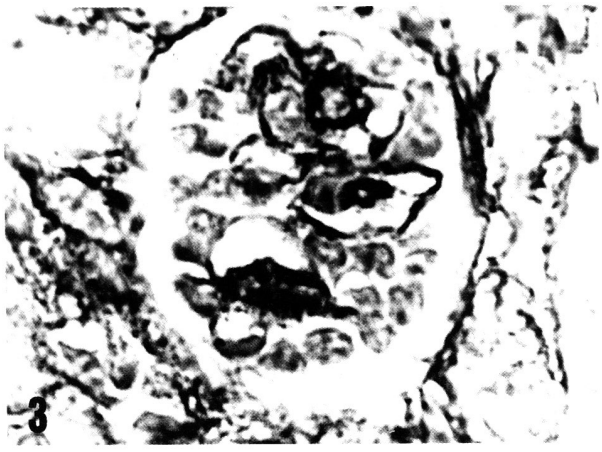
TN expression in developing renal corpuscle

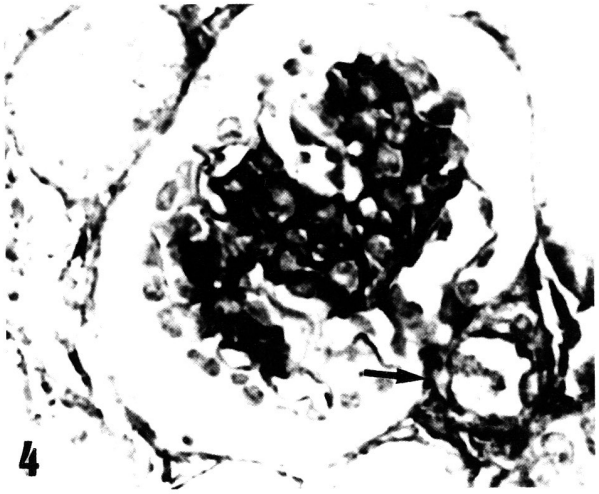
S-Shaped Body Stage. The renal corpuscle anlage was represented by the epithelium located around the narrow cleft in the outer aspect of the S-shaped body (Fig. 1). This cleft is where the capillary loops and mesangium develop in later stages.

In this stage, no cells and no TN expression were observed in the cleft of the S-shaped body (Fig. 1).

Developing Capillary Loop Stage. This stage was characterized by the presence of several capillary loops and by intercellular spaces between the developing podocytes (Fig. 2). In this stage, mesangial cells were observed for the first time.







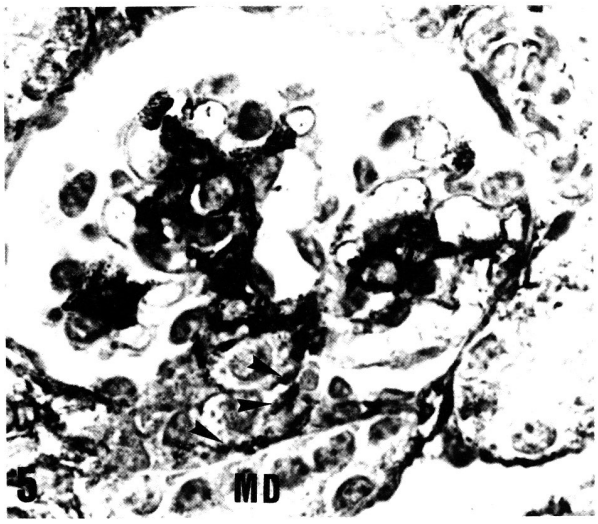


Fig. 1.- S-shaped body stage. A cleft (arrow) has appeared in the external surface of the wall. Note that only the ECM surrounding the S-shaped body shows TN reactivity. PE, parietal epithelium; VP, visceral epithelium; Asterisk, renal capsule. X 850.

Fig. 2.- Early developing loop stage. Note the presence of TN-positive material in the developing mesangium (arrowhead) and in the hilus zone (arrow). X 630.

Fig. 3.- Late developing loop stage. Several capillary loops can be recognized. Note the spots of strong TN reactivity in the mesangial area. X 630.

Figs. 4 and 5.- Maturing renal corpuscle stage. Multiple capillary lumina are evident. Note that the glomerular mesangial core is strongly labeled, but that labeling also extends to the afferent arteriole (arrow) and to the juxtaglomerular region (arrowheads). MD, macula densa. X 850.

Fig. 6.- Mature renal corpuscle stage. Note the strong labeling of the mesangial matrix. X 850.

TN expression in the mesangial matrix was first seen early in this stage as a weak staining (Fig. 2), which became markedly more intense as the complexity of the capillary loops increased (Fig. 3). In addition, TN antibody labeled the fibrillar ECM located close to the hilus of the developing renal corpuscle (Figs. 2 and 3). This labeling was stronger and occurred earlier than in the mesangial matrix.

Maturing Renal Corpuscle Stage. This stage was characterized by the presence of multiple capillary loops, but the podocytes had not yet achieved their mature, more flattened configuration (Figs. 4 and 5).

The mesangial cells appeared embedded in a strongly TN-immunoreactive matrix (Figs. 4 and 5), and the ECM surrounding the afferent (developing juxtaglomerular cells) and efferent arterioles was strongly TN-reactive (Figs. 4 and 5).

Mature Renal Corpuscle Stage. The podocytes, the parietal epithelium, and the endothelium appeared flattened. In this stage, the mesangial ECM was strongly labeled and occupied an extensive area (Fig. 6).

Discussion

The present results show that TN expression in the mesangial matrix first occurred very early in the developing renal corpuscle. The levels of expression increased progressively from the early developing capillary loop stage to the end of the maturing renal corpuscle stage. The high levels persisted in the mature renal corpuscles. This finding supports the notion that in the mouse kidney, as in the kidneys of other mammals (Truong et al., 1994a), TN is a component of normal mesangial matrix.

The origin and development of mesangial cells remain unclear. The major obstacle till now has been a lack of specific markers for mesangial cells. However, recently Lindahl et al. (1998) used the expression of platelet-derived growth factor (PDGF-Rb), desmin, and smooth muscle a-actin as markers and demonstrated that mesangial cells in the mouse kidney are present in the core of the tuft from the early developing capillary stage. These authors also proposed a model in which mesangial cells originate from progenitor cells surrounding the developing afferent and efferent arterioles. This model is consistent with the observation that, in the mature renal corpuscle, injured mesangial cells are repaired after migration of progenitor cells located near the juxtaglomerular apparatus (Hugo et al., 1997). The present study shows that TN expression in the hilus of the developing renal corpuscle precedes expression in the mesangial area. This finding is in keeping with the model proposed by Lindahl et al. (1998) to explain the origin of mesangial cells.

The appearance of TN expression and the presence of mesangial cells in the developing renal corpuscle occur at the same time. This suggests that the mesangial cells are the likely source of mesangial TN. This is in accordance with reported observations by Truong et al. (1994b) that TN is synthesized by mesangial cells in culture. Furthermore, the presence of TN-reactive ECM in the hilus of the developing renal corpuscle and in developing afferent and efferent arterioles, areas where the mesangial cell precursors are located, suggests that these cells are also able to synthesize TN.

A fundamental question arising from the close temporal relation between the start of TN expression and mesangial cell development is whether TN is necessary for mesangial cell development and differentiation. The present study could not answer this question. In the kidney of TN knockout mice, mesangial cells developed normally (Nakao et al., 1998). However, this evidence is not conclusive since other TN-like molecules might compensate for TN (Saga et al., 1992). The role of TN in the development and differentiation of mesangial cells requires further investigation.

Finally, it is worth emphasizing that TN is detected only in the mesangial matrix within the renal corpuscle. This suggests that TN may constitute a useful marker to study the development and maturation of mesangium.

Acknowledgements

I am grateful to Ian A. Williams for revising the English manuscript. This work was supported by grant 15/99 from Fundación Marqués de Valdecilla, and by grant PB98-1418-CO2-O2 From Dirección General de Enseñanza Superior e Investigación.

References

Aufderheide E, Chiquet-Ehrismann R and Ekblom P (1987). Epithelial-mesenchymal interactions in the developing kidney lead to expression of tenascin in the mesenchyme. *J Cell Biol,* 105: 599-608.

Chiquet M (1992). Tenascin: an extracellular matrix protein involved in morphogenesis of epithelial organs. *Kidney Int,* 41: 629-631

Chiquet-Ehrismann R (1995). Tenascins, a growing family of extracellular matrix proteins. *Experientia,* 51: 853-862.

Du Bois AM (1969). The embryonic kidney. In: Rouiller C, Muller AF (eds). The kidney: Morphology, Biochemistry, Physiology. Vol. I. Academic Press, New York, pp. 6-23.

Erickson HP (1993). Tenascin-C, tenascin-R, and tenascin-X- a family of talented proteins in search of functions. *Curr Opin Cell Biol,* 5: 869-876.

Hay ED (1991) Collagen and other matrix glycoproteins in embryogenesis. In: Hay ED (ed). *Cell Biology of extracellular matrix.* Plenum Press, New York, pp. 419-456.

HUGO C, SHANKLAND SJ, BOWEN-POPE DF, COUSER WG and JOHNSON RJ (1997). Extraglomerular origin of mesangial cell after injury. A new role of juxtaglomerular apparatus. *J Clin Invest,* 15: 786-794.

KOUKOULIS GK, GOULD VE, BHATTACHARYYA A, GOULD JE, HOWEEDY AA and VIRTANEN I (1991). Tenascin in normal, reactive, hyperplastic, and neoplastic tissue: Biological and pathologic implications. *Hum Pathol,* 22: 636-643.

LINDAHL P, HELLSTRÖM M, KALÉN M, KARLSSON L, PEKNY M, PEKNA M, SORIANO P and BETSHOLTZ C (1998). Paracrine PDGF-B/PDGF-Rb signaling controls mesangial cell development in kidney glomeruli. *Development,* 125: 3313-3322.

MARTINEZ-HERNANDEZ A and AMENTA PS (1983). The basement membrane in pathology. *Lab Invest,* 48: 656-677.

NAKAO N, HIRAIWA N, YOSHIKI A, IKE F and KUSAKABE M (1998). Tenascin-C promotes healing of Habu-snake venom-induced glomerulonephritis: studies in knockout congenic mice and in culture. *Am J Pathol,* 152: 1237-1245.

OJEDA JL, ROS MA and ICARDO JM (1989). A technique for fluorescence microscopy in semithin sections. *Stain Technol,* 64: 243-248.

OJEDA JL, ROS MA and ICARDO JM (1993). Lectin-binding sites during postnatal differentiation of normal and cystic rabbit renal corpuscles. *Anat Embryol,* 187: 539-547.

REEVES W, CAULFIELD JP and FARQUHAR MG (1978). Differentiation of epithelial foot processes and filtration slits. Sequential appearance of occluding junctions, epithelial polyanion, and slit membranes in developing glomeruli. *Lab Invest,* 39: 90-100.

SAGA Y, YAGI T, IKAWA Y, SAKAKURA T and AIZAWA S (1992). Mice develop normally without tenascin. *Genes Dev,* 6: 1821-1831.

TRUONG LD, PINDUR J, BARRIOS R, D'AGATI V, LECHAGO J, SUKI W and MAJESKY M (1994a). Tenascin is an important component of the glomerular extracellular matrix in normal and pathologic conditions. *Kidney Int,* 45: 201-210.

TRUONG LD, MAJESKY MW and PINDUR J (1994b) Tenascin is synthesised and secreted by rat mesangial cells in culture and is present in extracellular matrix in human glomerular diseases. *J Am Soc Nephrol,* 4: 1771-1777

TRUONG LD, PINDUR J, FOSTER S, MAJESKY M and SUKI WN (1996). Tenascin expression in nephrogenesis and in normal or pathologic glomerulus morphologic features and functional implications. *Nephron,* 72: 499-506.