IDENTIFICATION OF COLLAGEN TYPE IV IN THE GLOMERULUS OF THE ATLANTIC HAGFISH MYXINE GLUTINOSA

Ute Eisenberger, Lena Koob-Emunds, Hilmar Stolte
Laboratory of Experimental Nephrology, Division of Nephrology, Department of Internal Medicine, Medical School Hannover, Germany

The glomerulus of Myxine glutinosa is a valuable model to study renal filtration barrier function (Fels L.M. et al., Renal. Physiol. Biochem. 16:276-84, 1993). In recent investigations, we focused on extracellular matrix proteins, like fibronectin (Kastner S. et al., Bull. MDIBL 30: 122, 1991) and proteoglycans (Fels L.M. et al., Bull. MDIBL this issue), and their role in pathomechanisms and glomerular functional changes. To further elucidate the pathophysiology of glomerular basement membrane (GBM), it is important to identify other structural proteins in hagfish. A basic constituent of the GBM in the mammalian kidney is collagen type IV. This glycoprotein (M_r of 550000 - 600000) has a unique network-forming structure in comparison to other collagens, providing a microskeleton for the attachement of other GBM components (Dousa T.P., In: Seldin D.W., Giebich G.,ed. The kidney: Physiology and Pathophysiology, Plenum Press, New York, chap. 27, p. 645-667, 1985). Subsequently, this investigation was focused on the identification of collagen IV in Myxine glutinosa.

Glomeruli of Myxine glutinosa were dissected and sonicated. The homogenate was extracted with 8 M guanidin-HCl, 50mM Na-acetate at pH 6 for 14 h. By dialysing the supernatant against H_2O, a protein precipitate was formed. In SDS-PAGE followed by Coomassie brilliant blue staining, a matching band in precipitate and collagen type IV standard from human placenta was observed at 120 kD under reducing conditions and was verified as collagen by an additional collagenase digestion (Type IV, Sigma, St. Louis, USA) (fig.1). In a second approach, a glomeruli homogenate was digested by collagenase (0.002 mg collagenase/1 mg homogenate) (modified after Wieslander et al. J., Biol. Chem. 260:8564-70, 1985) and then dialysed against 0.05 M $NaHCO_3$. The supernatant was applied to SDS-PAGE and immunoblotting was performed with a polyclonal α_1-NC1 antibody (a gift from M. Slade, Kings College, London). This procedure revealed a specific band at approximatly 28 kD, which indicates the presence of α_1-NC1-monomers in the glomeruli homogenates (fig.2).

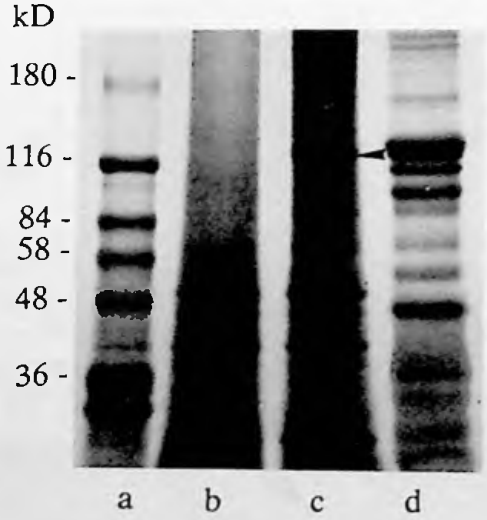

fig.1: SDS-PAGE of guanidin-extracted glomeruli homogenates of Myxine glutinosa

lane a. molecular marker ; lane b. guanidin-extracted glomeruli homogenate with digestion / lane c. without collagenase digestion; lane d. collagen type IV standard from human placenta;
arrow identifies collagen band in the glomeruli homogenate at 120 kD;

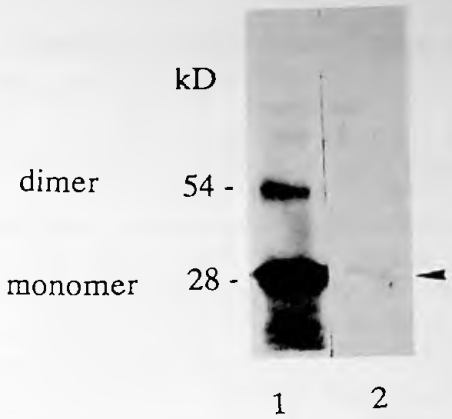

fig.2 Immunoblotting against α_1-NC1 of Collagen Type IV of glomeruli homogenates of Myxine glutinosa (Western blot)

lane 1. NC1 standard from bovine kidney; lane 2. supernatant of collagenase digested glomeruli homogenate with specific band at 28 kD (arrow);

In summary, this study confirmed our hypothesis, that collagen type IV occurs in the glomerulus of Myxine glutinosa. By immunoblotting, we could identify the α_1-NCI subunit, indicating the presence of the α_1-chain of collagen type IV in the atlantic hagfish. According to investigations in insects, the α_1-chain seems to be the ancestor for the five subtypes of α-chains known today (Timpl R., Eur. J. Biochem. 180:487-502, 1989; Johansson C. et al., J. Biol. Chem. 267: 24533-537, 1992).

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