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Nephron induction – the epithelial mesenchymal interface revisited

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Abstract While more and more humoral factors are being implicated in nephrogenesis, there is no detailed knowledge of the morphological structures at the interface of the nephron inducer and the surrounding mesenchyme. Hence we examined this area in the cortex of neonatal rabbit kidneys by scanning and transmission electron microscopy. Our interest was focused on the basal aspect of the collecting duct ampulla and the surrounding competent mesenchyme where morphogenic signals are exchanged during nephron induction. Close contact between these two tissues is assumed during nephrogenesis to allow direct cellular contact or diffusion of soluble factors across a short distance. However, our data show the presence a wide cleft around the collecting duct ampulla spatially separating the inducer and the competent mesenchyme during nephron induction. This cleft is filled with a characteristic fibrillar meshwork.

Keywords Kidney development · Nephron induction · Extracellular matrix · Electron microscopy

Introduction

Despite an intensive analysis of gene expression and cellular interaction during early kidney development, the primary morphogenic stimulus for nephron formation is yet to be identified [1]. During nephron induction, morphogenic information must be transmitted at the basolateral aspect of the epithelial cells [2]. Currently one hypothesis is the release of humoral factors by the epithelial cells of the ampullar tip, which diffuse across the extracellular space and exert a morphogenic function on competent mesenchymal cells. This view is backed by in vitro experiments in which metanephrogenic mesenchyme could be induced by conditioned medium from a ureteric bud cell line [1]. Another possibility is the direct exchange of morphogenic signals following the formation of temporary cell-cell contacts between epithelial cells of the collecting duct ampulla and the surrounding mesenchyme. This hypothesis is backed by in vitro transfilter experiments [3, 4]. In these experiments a filter with a defined pore size separated inducer and competent mesenchyme. Nephron induction only occurred when the filter pores were large enough to allow the formation of cell-cell contacts between both tissues.

The exchange of humoral factors as well as the formation of cell-cell contacts would require a close and intensive contact of epithelium and surrounding competent mesenchyme. Humoral factors would be most effective across a short diffusion distance, because the mesenchyme displays only a relatively short competence window [4]. The formation of cell-cell contacts would definitively require both tissues to be in direct contact. Surprisingly very little information is available on the epithelial-mesenchymal interphase. The only morphological data available are from an electron-microscopic study of embryonic mouse kidneys performed by Lehtonen in 1975 [5], which demonstrated ruthenium red-positive material around the collecting duct ampulla.

Hence we performed a detailed morphological study of this region. This morphological analysis showed striking structural characteristics of the collecting duct ampulla not described to date. In our opinion these structures could play a crucial role in nephron induction.

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Materials and methods

Tissue preparation

Newborn New Zealand rabbits were anesthetized with ether and killed by cervical dislocation. Both kidneys were removed immediately and cut precisely along the corticomedullary axis.

Immunogold pre-embedding for electron microscopy

Cryosections (20-μm) of neonatal rabbit kidneys were fixed in 0.02% glutaraldehyde and washed in phosphate-buffered saline

(PBS). The sections were then incubated in blocking solution (PBS containing 1% bovine serum albumin and 10% horse serum) for 30 min. Undiluted monoclonal antibody $PCD_{Amp}1$ [6] was applied for 90 min and the sections were washed again in PBS. A 5- to 6-nm gold-conjugated species-specific secondary antibody (Aurion, Wageningen, Netherlands) was applied for 45 min at a dilution of 1:10. Following a final wash, the sections were dehydrated in a graded series of ethanols. The sections were critical-point dried in carbon dioxide, carbon coated (Balzers, Liechtenstein, Germany), and examined in a DSM 940 A scanning electron microscope (Zeiss, Oberkochen, Germany) using a BSE detector (Zeiss).

Transmission electron microscopy

For transmission electron microscopy small pieces of freshly prepared tissue were immediately fixed in 2% paraformaldehyde/2.5% glutaraldehyde in 0.1 M cacodylate buffer (12 h, 4°C), post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer, and block contrasted in 1% uranyl acetate in maleate buffer. The tissue was then dehydrated in a graded series of ethanols and embedded in Epon (polymerized at 60°C for 48 h). Ultrathin sections were cut with a glass knife on an OmU3 ultramicrotome (Reichert, Vienna, Austria) and then transferred to 200 lines/inch mesh nickel grids (SCI, Munich, Germany). Following a contrasting step with 4% uranyl acetate/lead citrate, the sections were examined in an EM 902 transmission electron microscope (Zeiss). Semithin sections for light microscopy were stained with Richardson solution and analyzed in a Zeiss Axiovert 35 (Zeiss) in bright field.

Scanning electron microscopy

For scanning electron microscopy exactly oriented pieces of tissue were prepared, fixed in 2% glutaraldehyde in PBS under isotonic conditions (24 h, 4°C), dehydrated in a graded series of ethanols, transferred to acetone, and critical point dried in carbon dioxide. Finally they were sputter coated with gold (Polaron, Watford, UK) and examined in a DSM 940 A scanning electron microscope (Zeiss).

Results

In all micrographs of embryonic kidney analyzed by our laboratory and by other authors [4] a clear cleft was visible at the basal aspect of the collecting duct ampulla (Fig. 1a, b). However, this obvious cleft has not been described or studied in detail to date.

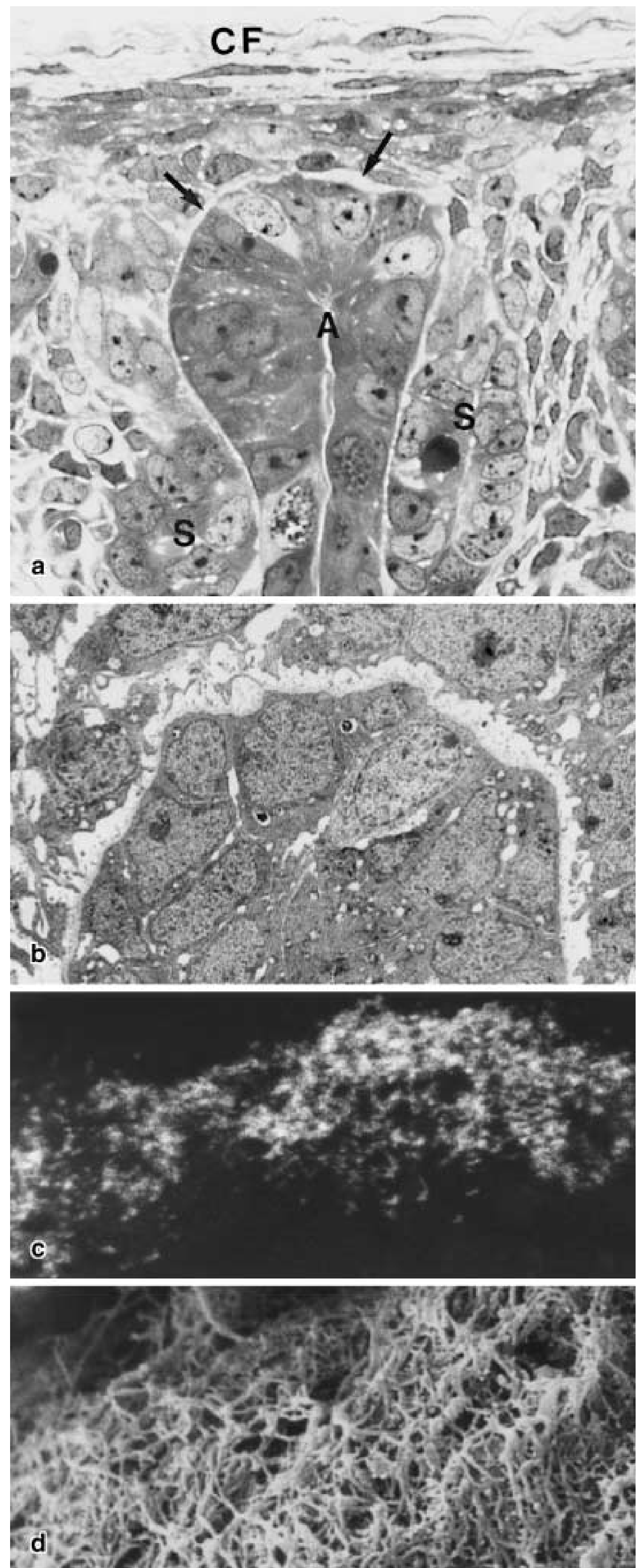

Fig. 1 **a** Embryonic collecting duct ampulla in the cortex of neonatal rabbit kidney. Light micrograph of semithin section. The ampulla (*A*) directly underneath the capsula fibrosa (*CF*) is surrounded by a clear cleft (*arrows*). *S*, developing nephron; magnification x575. **b** Transmission electron micrographs of the basal aspect of the ampullar tip**.** A wide cleft filled with extracellular matrix surrounds the basal aspect of the collecting duct ampulla separating epithelium and mesenchyme; magnification x1,400. **c** Ultrastructural localization of $P_{CD}Amp1$ at the tip of the collecting duct ampulla. Immunogold pre-embedding incubation with monoclonal antibody $P_{CD}Amp1$. Scanning electron micrograph, backscattered electron image (BSE) of a collecting duct ampulla. The basement membrane of the embryonic collecting duct ampulla (A) is clearly labelled. The immunolabel appears as a wide band resembling a densely woven meshwork; magnification x8,200. **d** Scanning electron micrograph of the basal aspect of the collecting duct ampulla. The basal aspect of the tip of the collecting duct ampulla is covered with a dense fleece. This extracellular fleece is made up from a meshwork of various fibers differing in thickness and structure; magnification x9,800

Electron-microscopic analysis showed cell processes on many mesenchymal cells reaching into the cleft towards the collecting duct ampulla. However, despite a systematic search in many consecutive sections, cell-cell contacts between these mesenchymal cell processes and epithelial cells could not be found (Fig. 1b).

The transmission electron-microscopic analysis of the cleft area revealed a partially developed basement lamina with a number of discontinuities at the epithelium of the ampullar tip, as already described by Saxén [4] and Lehtonen [5]. In immunogold electron-microscopic studies using a monoclonal antibody against the matrix-associated $PCD_{Amp}1$ [6], we found a lamina fibroreticularis averaging 1.6 μm in thickness and consisting of thick fibers and a variety of thinner branching fibers (Fig. 1c). This lamina fibroreticularis completely filled the interstitium surrounding the collecting duct ampulla.

Scanning electron micrographs showed the dense fibrous material of the lamina fibroreticularis surrounding the collecting duct ampulla and confirmed the existence of a strikingly thick fleece-like basement membrane in this region (Fig. 1d). This fleece was most prominent at the ampullar tip and decreased in thickness along the ampullar neck region. The fibrous network was characteristic for the collecting duct ampulla. Nephron tubules showed a continuous basement lamina with no lamina fibroreticularis and displayed a completely smooth basal surface in the scanning electron microscope.

Discussion

Our studies could not confirm the close contact between the collecting duct ampulla and the surrounding mesenchyme assumed to date, making it more difficult to imagine how morphogenic information could be exchanged during nephron induction. A humoral factor would have to diffuse across a wide interstitium with an unexpectedly large volume. Although still possible, this would require the release of a rather large amount of such a morphogenic factor in order to reach the distant mesenchymal cells.

However, the transport of morphogenic information across the cleft by direct cell-cell contacts would require long mesenchymal cell processes to cross the cleft and to come into contact with the basal aspect of the collecting duct epithelium. Despite a systematic search, such processes could not be demonstrated.

The fibers of the pericellular fleece around the collecting duct ampulla form the only structural connection between the nephron inducer and the competent mesenchyme. Many mesenchymal cells display short processes reaching into the cleft to be in contact with the pericellular matrix. These findings lead us to the hypothesis that mesenchymal cells could receive morphogenic information by contact with the extracellular matrix. Barasch et al. [2] postulated a diffusion-limited molecule at the basolateral aspect of the collecting duct epithelium as a morphogenic stimulus for nephrogenesis. This molecule is likely to be bound to the basal plasma membrane or within the basement membrane of the collecting duct ampulla.

One possible source of morphogenic information within the extracellular matrix is signal sequences that can transmit information by binding to transmembrane receptors. Amino acid sequences, such as RGD sequences, that exert specific signalling functions have been described for a number of extracellular matrix components [7]. Extracellular matrix receptors such as integrins [8] are examples of the transmission of signals from the extracellular matrix into a cell. A signal transduction cascade coupled to the actin cytoskeleton [9], paxillin [10], or protein tyrosine kinases, such as focal adhesion kinase [11], could launch a morphogenic program by initiating the transcription of specific genes.

The importance of signal transduction by the extracellular matrix has definitely been underestimated [12]. An essential role of the extracellular matrix in nephron induction would explain the lack of success in isolating a primary factor for nephron induction, despite years of intensive search.

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