The Journal of Maternal-Fetal and Neonatal Medicine, 2012; 25(S5): 72–75

ISSN 1476-7058 print/ISSN 1476-4954 online
DOI: 10.3109/14767058.2012.714645

informa
healthcare

The pine-cone body: an intermediate structure between the cap mesenchyme and the renal vesicle in the developing nod mouse kidney revealed by an ultrastructural study

Marco Piludu[1], Vassilios Fanos[2], Terenzio Congiu[3], Monica Piras[1], Clara Gerosa[4], Cristina Mocci[4], Daniela Fanni[4], Sonia Nemolato[4], Sandro Muntoni[1], Nicoletta Iacovidou[5] & Gavino Faa[4]

[1]Department of Biomedicine, University of Cagliari, Cagliari, Italy, [2]Department of Surgery, Section of Neonatal Intensive Care Unit, Puericulture Institute and Neonatal Section, University of Cagliari, Cagliari, Italy, [3]Department of Surgical and Morphological Sciences, University of Insubria, Varese, Italy, [4]Department of Surgery, Division of Pathology, University of Cagliari, Cagliari, Italy, and [5]Department of Obstetrics and Gynaecology, Aretaieion Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece

Nephrogenesis is mainly characterized by the interaction of two distinct renal constituents, the ureteric bud and the metanephric mesenchyme. In this paper we describe by means of light and electron microscopic techniques the morphological events that take place during the early stages of cap mesenchymal formation. Samples of normal renal tissue were excised from newborn NOD mice and processed by standard light and electron microscopy techniques. In all samples examined we detected the presence of several cap mesenchymal aggregates in different stages of differentiation. They varied from small solid nodules with few ovoid cells to bigger pine-cone-like aggregates, characterized by a peculiar distribution and morphology of their cellular constituents. Our data highlight, for the first time, the presence of a specific cap mesenchymal structure, the pine-cone body and show, at ultrastructural level, how each cap aggregate epithelializes proceeding in stages from a condensed mesenchymal aggregate to the renal vesicle, through the intermediate "pine-cone body" stage.

Keywords: Electron microscopy, glomerulogenesis, nephrons, nephrogenesis

Introduction

The mature kidney of mammals is the final product of three embryonic excretory organs: the pronephros, the mesonephros and the metanephros [1]. The metanephros takes origin from two main components: the ureteric bud (UB), a branching epithelial tube originating from the Wolffian duct, and the mesenchymal cells of the metanephric mesenchyme, that originate from the intermediate mesenchyma [2]. The metanephric mesenchymal cells are programmed to make epithelial precursors of the mature kidney in response to inductive signals deriving from the UB cells [3]. While epithelial cords originating from the UB are branching into the metanephric mesenchyme, some self renewing progenitor cells condensate and aggregate around the tips of UB-derived epithelial branches, transforming themselves into the cap mesenchyme [4–6]. The subsequent steps of nephron development are characterized by the mesenchymal-to-epithelial transition of cap mesenchymal cells, which will form most of the epithelia of the mature human kidney [7,8]. Since, at the best of our knowledge, no extensive studies have been reported on the fine structure of cap mesenchymal cells in the early phases of their origin from the metanephric mesenchyme, and during their transition towards the epithelial phenotype, the aim of this study was to acquire, by means of transmission electron microscopy, further ultrastructural informations concerning the specific morphological events occurring during the early stages of cap mesenchymal development and differentiation.

Material and methods

Five newborn non obese diabetic (NOD) mice were utilized in this study. The animals were obtained from a local colony housed in a pathogen free environment in the animal care facility of the University of Cagliari (Cittadella Universitaria di Monserrato), where all the breeding conditions were well controlled. According to the guidelines for the Care and Use of Laboratory Animals (NIH) and the European Communities Council Directive for the use of animals in scientific experiments, the mice were anesthetized and euthanized. No evident pathology was observed in any of the animals used in this study. The kidneys were then excised, cut into small pieces, fixed in a mixture of 3% formaldehyde and 0.1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, rinsed and stored in 0.1 M cacodylate buffer at 4°C. The samples were processed by standard methods for embedding in LR gold resin (Fluka Biochemica). In detail, they were dehydrated in a cold graded methanol series and infiltrated in LR gold resin. After infiltration, the specimens were transferred in gelatin capsules or in flat polyethylene molds filled with fresh resin and placed in a polymerization chamber under UV light (365 nm) at −20°C. For light microscopic observations one micrometer sections were cut and collected on superfrost slides, stained with toluidine blue, observed and photographed in a Leica 2000 microscope. For electron microscopic investigations, ultrathin sections were cut with diamond knife, collected on formvar-coated 100-mesh grids, stained with uranyle acetate and bismuth subnitrate, observed and photographed in a JEOL 100 S transmission electron microscope (TEM).

Correspondence: Prof Gavino Faa, Department of Surgery, Institute of Pathology, University of Cagliari, Cagliari, Italy. E-mail: gavinofaa@gmail.com

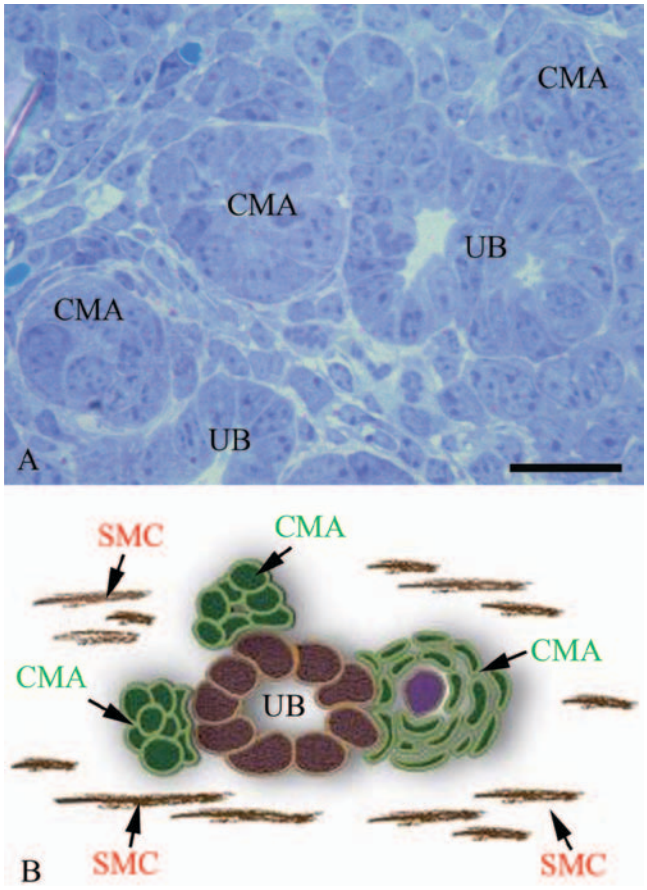

Figure 1. The complex scenery of early phases of nephrogenesis in the NOD mouse kidney. (A) Light micrograph of the mouse renal cortex showing active nephrogenesis. Ureteric buds (UB) are surrounded by cap mesenchymal aggregates (CMA) that differ in size and shape. Bar = 20 μm. (B) Schematic representation of the ureteric bud (UB) and the adjacent cap mesenchymal aggregates (CMA) among scattered mesenchymal cells (SMC) in the developing mouse renal cortex.

Results

We focused our analysis on the outer portions of renal cortex where both light and electron microscopy showed active nephrogenesis. In the subcapsular regions, scattered and isolated mesenchymal cells, remnants of the metanephric mesenchyme, appeared intermingled with the cap mesenchymal nodules, condensed cellular solid aggregates roundish or ovoid in shape. At low power, two or more cap mesenchymal aggregates were frequently found to surround a single ureteric bud's branch (Figure 1). Cap mesenchymal aggregates showed evident variability in their morphological appearance and size, varying from small cellular solid nodules comprising few ovoid cells to bigger aggregates with a conspicuous number of cells (Figure 1). At higher magnification, electron microscopy emphasized the fine structure of cell organization and architecture in the different cap mesenchymal aggregates, showing details quite beyond those expected from the resolving power of the light microscopy (Figures 2 and 3). All cells present in cap mesenchymal nodules exhibited peculiar morphological features. In general, they were characterized by a small cell body with a scanty cytoplasm containing few cellular organelles and few mitochondria; the nucleus was large, often showing prominent nucleoli (Figures 2 and 3). The nucleoli displayed a peculiar morphology, being often characterized by variations in their size, shape and number (Figures 2 and 3). Moreover, cap mesenchymal cells exhibited different affinity to bismuth subnitrate and uranile acetate staining, so that some of them resulted more consistently labeled within the same cap mesenchymal aggregate (Figure 3).

Interestingly, while some solid cap mesenchymal nodules exhibited an omogenous population of few roundish cells (Figure 3A), other cap aggregates showed an evident degree of variability in shape and morphology of their cells (Figure 3B and 3C). Whereas the center of the cap aggregates was occupied by a roundish cell, in their outer regions they were characterized by the presence of thin curved shaped cell types, that seemed to twist around a fixed central cluster, resembling a pine-cone-shaped structure (Figure 3B and 3C). Apoptotic cells and mitotic figures were often observed in all kidney samples analyzed in this study.

Discussion

Early studies on nephrogenesis have speculated the sequence of morphological events that lead to glomerulogenesis and tubulogenesis [4]. The process might start with the outgrowth of the primary nephric duct and the ureteric bud, which invades the metanephric mesenchyma and induce the differentiation of the renal epithelial precursors [8]. According with this hypothesis, the final stages could be characterized by the maturation of glomeruli and by their progressive migration toward mid and deep cortex [3,4,9]. Most of the previous studies regarding renal development were carried out by means of light microscopy [10–13], whereas only few electron microscopic investigations were reported [14–17]. No doubt that the higher magnification of the electron microscopy techniques that we used in this study has revealed ultrastructural details beyond the resolving power of the light microscopy, highlighting the morphological changes that occur during the early stages of cap mesenchymal differentiation. In this study we showed changes in the morphological appearance of the cap mesenchymal aggregates, varying from small solid nodules with few ovoid cells to bigger pine-cone-like aggregates. It is reasonable to assume that similar changes in the size and appearance of developing renal cells may be correlated to the various stages of cellular differentiation that take place during cap mesenchymal development. In growing cap aggregates, the evident changes of the cellular shape revealed in this study, as well as the different cellular affinity to bismuth subnitrate and uranile acetate staining, could be related to the maturation and aging of their cells. It's conceivable to assume that most of the curved cells detected in the outer regions of the cap mesenchymal aggregates evolve from the ovoid cells that were usually found in the central area of the same aggregate. It's generally believed that variations of the cellular shape modify the area contact between adjacent cells and could account for changes in transmembrane signaling and consequently for changes of cellular metabolic activity [18,19].

A chaotic multitude of renal cells in different states of cellular metabolic activity characterized the fascinating scenery of the cap mesenchymal formation. Mitotic figures and apoptotic cells were often seen to coexist with metabolically active cap mesenchymal cells characterized by the presence of prominent and pleomorphic nucleoli. It's well known that the presence of well developed nucleoli reflects RNA synthesising and therefore protein synthesizing that is generally assumed to reflect metabolically active cells [20]. Complexity and enlargement of nucleolar form were previously observed in tumor cells by several authors [21,22] and were reported in proliferating cells, including cells in tissue culture, stem cells and embryonic cells [23]. Moreover, multiple nucleoli

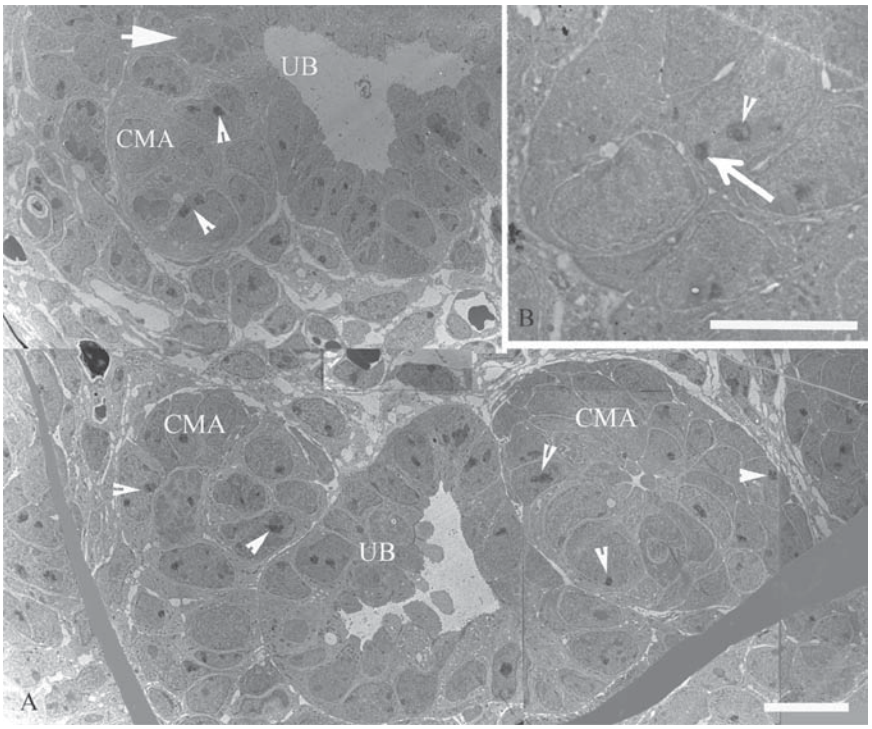

Figure 2. Electron micrographs showing at higher magnification the outer portion of the mouse renal cortex. (A) Cap mesenchymal aggregates (CMA) adjacent to the ureteric buds (UB) are present in different stages of differentiation. (B) Detail of a cap mesenchymal aggregate. Nucleoli (Arrowheads). Mitotic figure (Arrow). Bars = 10 μm.

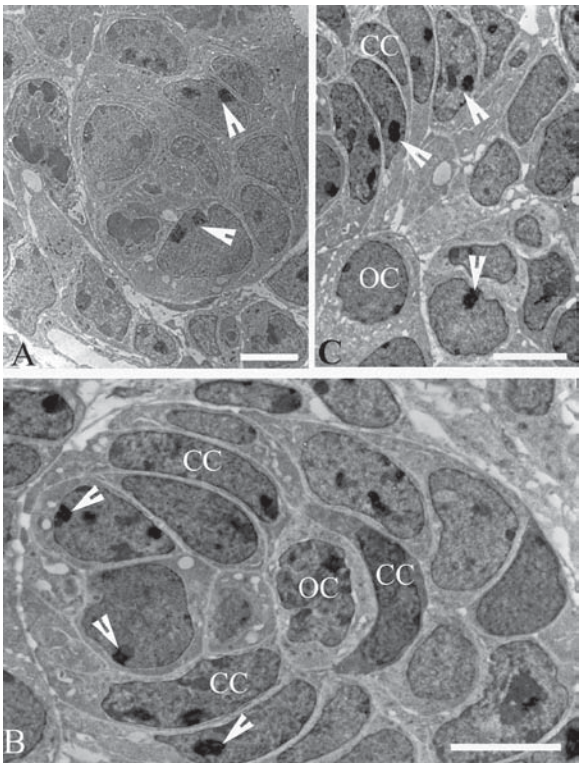

Figure 3. Details of cap mesenchymal aggregates. (A) Small cellular solid nodule comprising few ovoid cells. (B, C) "Pine-cone body" characterized by a more conspicuous number of cells. Note the presence of the ovoid cell (OC) in the central region surrounded by different thin curved shaped cells (CC), resembling a pine-cone-shaped structure. "Pine-cone body" cap mesenchymal cells exhibit different affinity to bismuth subnitrate and uranile acetate staining. Note the presence of evident nucleoli (arrowheads) in most of the cellular constituents of the renal tissues. Bars = 5 μm.

were found in the growing oocytes of specific organisms where they were supposed "to build up a large reservoir of maternal ribosome in order to sustain early embryonic development" [24]. The prominent pleomorphic nucleoli may indicate a significant increased cellular metabolic activity associated with cellular differentiation during cap mesenchymal development. Taken all together, these findings suggest that the early inductive events that convert the mesenchyme to epithelium proceed in stages from the condensed mesenchymal nodule to the renal vesicle through the intermediate "pine-cone body" stage. Moreover they indicate that the entire cap developmental process represents the consequence of a complex balance between specific intercellular signals involved in the regulation of protein synthesis, cell proliferation, cell motility and apoptosis.

In conclusion, our study of nephrogenesis at ultrastructural level in the developing NOD mouse kidney shows, for the first time, the presence of a peculiar structure, here defined the "pine-cone body," due to its peculiar architectural organization of the elongated comma-shaped mesenchyma-to-epithelial cells around a central roundish cell. This finding highlights our ignorance of some of the important morphological changes that characterize cellular differentiation processes during early cap mesenchymal development. The observation of this new developmental structure points out that further morphological studies by transmission and by scanning electron microscopy are needed to better characterize the different renal developmental steps in several animal species [11] as well as in humans. The significance of the morphological changes here described in cap mesenchymal cells in the "pine-cone bodies" remains to be ascertained and requires additional ultrastructural, immunohistochemical and molecular studies, in order to better investigate the intimate significance of this new developmental structure.

***Declaration of Interest*:** The authors declare no conflict of interest.

References

1. Fanni DGC, Nemolato S, Mocci C, Pichiri G, Coni P, Congiu T, Piludu M, et al. "Physiological" renal regenerating medicine in VLBW preterm infants: could a dream come true? J Matern Fetal Neonatal Med in press.
2. Poladia DP, Kish K, Kutay B, Hains D, Kegg H, Zhao H, Bates CM. Role of fibroblast growth factor receptors 1 and 2 in the metanephric mesenchyme. Dev Biol 2006;291:325–339.
3. Dressler GR. Epigenetics, development, and the kidney. J Am Soc Nephrol 2008;19:2060–2067.
4. Faa G, Gerosa C, Fanni D, Monga G, Zaffanello M, Van Eyken P, Fanos V. Morphogenesis and molecular mechanisms involved in human kidney development. J Cell Physiol 2012;227:1257–1268.
5. Sariola H. Nephron induction revisited: from caps to condensates. Curr Opin Nephrol Hypertens 2002;11:17–21.
6. Rosenblum ND. Developmental biology of the human kidney. Semin Fetal Neonatal Med 2008;13:125–132.
7. Carroll TJ, Park JS, Hayashi S, Majumdar A, McMahon AP. Wnt9b plays a central role in the regulation of mesenchymal to epithelial transitions underlying organogenesis of the mammalian urogenital system. Dev Cell 2005;9:283–292.
8. Faa G, Gerosa C, Fanni D, Nemolato S, Monga G, Fanos V. Kidney embryogenesis: how to look at old things with new eyes. In: Fanos V, Chevalier RL, Faa G, Cataldi L, editors. Developmental Nephrology: from embryology to metabolomics. Quartu Sant'Elena: Hygeia Press; 2011. pp 23–45.
9. Horster MF, Braun GS, Huber SM. Embryonic renal epithelia: induction, nephrogenesis, and cell differentiation. Physiol Rev 1999;79:1157–1191.
10. Fanni D, Fanos V, Monga G, Gerosa C, Nemolato S, Locci A, Van Eyken P, et al. MUC1 in mesenchymal-to-epithelial transition during human nephrogenesis: changing the fate of renal progenitor/stem cells? J Matern Fetal Neonatal Med 2011;24 Suppl 2:63–66.
11. Gerosa C, Fanos V, Fanni D, Nemolato S, Locci A, Xanthos T, Papalois A, et al. Toward nephrogenesis in the pig kidney: the composite tubulo-glomerular nodule. J Matern Fetal Neonatal Med 2011;24 Suppl 2:52–54.
12. Faa G, Gerosa C, Fanni D, Nemolato S, Locci A, Cabras T, Marinelli V, et al. Marked interindividual variability in renal maturation of preterm infants: lessons from autopsy. J Matern Fetal Neonatal Med 2010;23 Suppl 3:129–133.
13. Faa G, Gerosa C, Fanni D, Nemolato S, Marinelli V, Locci A, Senes G, et al. CD10 in the developing human kidney: immunoreactivity and possible role in renal embryogenesis. J Matern Fetal Neonatal Med 2012; 25:904–911.
14. Friis C. Postnatal development of the pig kidney: ultrastucure of the glomerulus and the proximal tubule. J Anat 1980;130:513–526.
15. Maric C, Ryan GB, Alcorn D. Embryonic and postnatal development of the rat renal interstitium. Anat Embryol 1997;195:503–514.
16. Dørup J, Maunsbach AB. The ultrastructural development of distal nephron segments in the human fetal kidney. Anat Embryol 1982;164:19–41.
17. Dørup J, Andersen GK, Maunsbach AB. Electron microscope analysis of tissue components identified and located by computer-assisted 3-D reconstructions: ultrastructural segmentation of the developing human proximal tubule. J Ultrastruct Res 1983;85:82–94.
18. Ben-Ze'ev A. Animal cell shape changes and gene expression. Bioessays 1991;13:207–212.
19. Ben-Ze'ev A. The role of changes in cell shape and contacts in the regulation of cytoskeleton expression during differentiation. J Cell Sci Suppl 1987;8:293–312.
20. Hernandez-Verdun D, Roussel P, Thiry M, Sirri V, Lafontaine DL. The nucleolus: structure/function relationship in RNA metabolism. Wiley Interdiscip Rev RNA 2010;1:415–431.
21. Busch H, Byvoet P, Smetana K. The nucleolus of the cancer cell: a review. Cancer Res 1963;23:313–339.
22. Busch H, Lane M, Adams HR, Debakey ME, Muramatsu M. Isolation of nucleoli from human tumors. Cancer Res 1965;25:225–233.
23. Zavala G, Vázquez-Nin GH. Analysis of nuclear ribonucleoproteic structures during notochordal cell differentiation and maturation in chick embryos. Anat Rec 2000;259:113–123.
24. Pederson T. Nucleolus, Overview. In: Lane WJLaMD, editor. Encyclopedia of Biological Chemistry. Elsevier; 2004. pp 119–122.