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Immunohistochemical markers of stem/progenitor cells in the developing human kidney

Alice Sanna[a,*], Vassilios Fanos[b], Clara Gerosa[a], Laura Vinci[a], Melania Puddu[b], Cristina Loddo[b], Gavino Faa[a]

[a] Division of Pathology, Department of Surgery, University of Cagliari, Cagliari, Italy
[b] NICU Center and Institute of Puericulture, Department of Surgery, University of Cagliari, Cagliari, Italy

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ABSTRACT

The aim of this study was to better define, by immunohistochemistry, the molecular markers of renal stem/progenitor cells localized in the different niches of ten human preterm kidneys with gestational age ranging from 11 up to 25 weeks. Our data evidence the existence of multiple stem/progenitor pools in different zones of the human developing kidney that are characterized by different phenotypes: capsular stem cells were EMA (MUC1)+, MDM2+, Vimentin+ and Wnt1+; progenitors of the sub-capsular nephrogenic zone were MDM2+ and Wnt1+; cap mesenchymal cells were EMA (MUC1)+, CD15+, vimentin+, Wt1+, CD10+, Bcl2+, Wnt1+ and PAX2+; interstitial progenitor cells were Vimentin+, Wt1+ and α1Antitripsin+. Our data evidence the existence of multiple stem/progenitor cell pools in the fetal and neonatal human kidney. Progenitors of these different pools are characterized by a peculiar phenotype, indicating a different differentiation stage of these renal progenitors. A better knowledge of the molecular markers expressed by renal stem/progenitors might represent a relevant datum for researchers involved in renal regenerative medicine.

Introduction

To this day, at least 8% of the European population (around 40 millions) suffers from chronic kidney disease (CKD) and this involves the risk of developing kidney damage (Hsu et al., 2004; Turin et al., 2012). When kidney disease progresses, it may eventually lead to kidney failure, which requires dialysis or a kidney transplant to maintain life. The current shortage of donor organs for adult patients need the development of new therapies for the treatment of CKD. Still, traditional stem cell-based approach has been demonstrated to be unable to regenerate the damaged organ. The increase in the incidence of CKD has been paralleled by the increased frequency of preterm births, reaching values of 12–13% in USA (Goldenberg et al., 2008), with the number of preterms for year being estimated around 15 million (Blencowe et al., 2013). Even though to date no conclusive evidence of any specific risk factor surely represents the cause of this epidemic of CKD (Remuzzi and Perico, 2014), the association of CKD and prematurity observed in multiple epidemiologic studies reinforces the Barker's hypothesis on the developmental origins of adult health and disease (Barker, 2004), and in particular the origin early in life of a susceptibility to develop renal disease later in life (Maringhini et al., 2010). Our research program focused on low birth weight newborns, confirming that they are characterized by a reduced nephron number at birth and, as a consequence, are at increased risk of developing CKD in childhood or adulthood (Puddu et al., 2009). A possible linkage between oligonephronia at birth and the development of adult hypertension and progressive renal disease has been identified in the compensatory glomerular hypertrophy. This morphological finding might constitute a risk factor that, during the years, increases the susceptibility of "forced" glomeruli to undergo exhaustion, ending with progression toward CKD (Brenner and Chertow, 1994). Focusing our attention on the preterm kidney, we were particularly attracted to better define the complexity of stem/progenitors localized in the different niches of the preterm kidney, that could represent the target of a potential regenerative therapy in the first weeks of the postnatal life. This work was aimed

Abbreviations: Bcl2, B-cell lymphoma 2; CD10, neprilysin; CD15, 3-fucosyl-*N*-acetyl-lactosamine; CD133, prominin 1; CKD, chronic kidney disease; CK7, cytokeratin 7; CK8-18, cytokeratin 8-18; EMA (MUC1), anti-endomysial (mucin 1); MDM2, mouse double minute 2 homolog; PAX2, paired box gene 2; Pecs, parietal epithelial cells; Wnt1, wingless-type MMTV integration site family member 1; WT1, Wilms tumor 1.

* Corresponding author.
E-mail address: alice.sanna85@tiscali.it (A. Sanna).

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Table 1
Antibodies utilized in this study.

Antibody	Dilution	Animal	Produced by
EMA (MUC1)	1:150	Mouse	Dako
Galectina 3	1:50	Mouse	Genetex
S100	1:2000	Rabbit	Dako
CD20	1:400	Mouse	Dako
Ki67	1:100	Mouse	Dako
HBME1	1:50	Mouse	Dako
Sinaptofisina	1:20	Mouse	Dako
CD79a	1:200	Mouse	Dako
Cromogranina a	1:300	Mouse	Dako
CD5	1:50	Mouse	Dako
Carletinina	1:50	Mouse	Dako
p16	1:50	Mouse	Santa Cruz
p63	1:50	Mouse	Sigma
CD21	1:50	Mouse	Dako
TTF1	1:100	Mouse	Dako
p53	1:50	Mouse	Dako
CK20	1:50	Mouse	Dako
Melan A	1:50	Mouse	Dako
CD23	1:500	Mouse	Dako
CD30	1:40	Mouse	Dako
CD3	1:200	Rabbit	Dako
Inibina α	1:50	Mouse	Dako
CD15	1:500	Mouse	Dako
Antibody	**Dilution**	**Animal**	**Produced by**
MDM2	1:10	Mouse	Santa Cruz
CK8-18	1:50	Mouse	Dako
CK7	1:150	Mouse	Dako
Glypican 3	1:200	Mouse	Dako
Vimentin	1:1000	Mouse	Dako
WT1	1:100	Mouse	Dako
α1 Anti-tripsina	1:2000	Rabbit	Dako
Nestin	1:200	Mouse	Santa Cruz
CD10	1:1000	Mouse	Dako
BCL2	1:150	Mouse	Dako
Wnt1	1:50	Rabbit	Abnova
PAX2	1:400	Mouse	Abnova
CD133	1:200	Rabbit	Abnova

at defining the molecular markers of renal stem/progenitor cells in the preterm infants by immunohistochemistry, in order to evidence the possible molecular targets for a "physiological" regenerative medicine (Fanni et al., 2012) to be started in the first postnatal weeks of life.

Materials and methods

Ten human fetuses with gestational age ranging from 11 up to 25 weeks were included in the present study. At macroscopy, no malformation were observed. Kidney samples were 10% formalin fixed, routinely processed and paraffin-embedded. Immunohistochemistry was performed on 4 μm-thick paraffin sections. Heat-induced antigen retrieval was carried out by steaming unstained sections in Target Retrieval Solution (Dako Denmark A/S, Glostrup, Denmark) for 30 min. The monoclonal and polyclonal antibodies utilized in this study, with their dilution are reported in Table 1. Both monoclonal and polyclonal antibodies were incubated for 20 min at room temperature. Sections were processed utilizing the labeled streptavidin–biotin complex system (LSAB2, Dako) in a Dako autostainer (DakoCytomation, Carpintera, CA, USA). As negative control samples, fetal kidney sections were incubated without antibody. Sections were counterstained with Mayer's hematoxylin. Immunoreactivity for all the antibodies was evaluated in the following compartments of the developing kidney: capsule, subcapsular zone, renal vesiscles, glomeruli (parietal epithelial cells, podocytes, mesangium), distal tubules, proximal tubules, collecting ducts and Henle's loop.

Results

The most relevant data regarding immunoreactivity of renal stem/progenitor cells and differentiated kidney cells in the fetal and neonatal kidneys analyzed in this study are reported in Table 2. No significant differences were detected in immunoreactivity for the utilized markers of the different renal stem/progenitor pools among subjects of different gestational age. Our data evidence the existence of multiple stem/progenitor pools in different zones of the human developing kidney. In order to better show the peculiar immunohistochemical patterns of different stem cell pools, they will be reported separately.

Capsule

The renal capsule appears as an important niche for stem/progenitors in the developing kidney at all the gestational ages here analyzed. At morphology, the capsular stem/progenitors did not show any differential marker when compared to fibroblast. At immunohistochemistry, stem/progenitor cells of the capsule were immunoreactive for EMA (MUC1) and Vimentin (Fig. 1a–b). Moreover, scattered small undifferentiated cells were detected in the capsule, characterized by a different phenotype, being reactive for MDM2 and Wnt1 (Fig. 2a–b).

Sub-capsular nephrogenic zone, also known as "blue strip"

The blue strip represents an aggregate of undifferentiated stem/progenitor cells occupying the subcapsular area. In this study, the phenotype of these stem/progenitors was characterized by immunoreactivity for MDM2 and Wnt1 (Fig. 2a–b).

Cap mesenchyme

The stem progenitors condensating around the ureteric bud tips that have been committed toward the epithelial fate, represent another compartment of renal progenitors. EMA (MUC1), CD15, Vimentin, WT1 (Fig. 3a), CD10, BCl2 (Fig. 3b), Wnt1 and PAX2 were expressed in the cap mesenchyme cells.

Renal vesicles

The renal vesicles are the first epithelial structures originating from the cap mesenchymal cells, representing an intermediate step in nephron formation. In the present study, cells giving rise to renal vesicles were immunostained by EMA (MUC1), CD15, CK8-18, Glypican 3, WT1, CD10, BCl2 (Fig. 4a), Wnt1 (Fig. 4b) and PAX2.

Interstitial cells

Scattered scarcely differentiated interstitial cells, often oval in shape, were immunoreactive for Vimentin (Fig. 5), WT1 and α1Anti-tripsin. This peculiar phenotype allowed their differentiation from interstitial histiocytes and fibroblasts

Parietal epithelial cells (Pecs)

Stem/progenitors were also detectable intermingled among the Pecs of the Bowman capsule in developing glomeruli. These progenitors located among Pecs were positive for multiple markers, including Vimentin, WT1, CD10 (Fig. 6a), BCl2 (Fig. 6b) and Wnt1.

Podocytes

Vimentin, WT1 (Fig. 7a), Nestin (Fig. 7b) and CD10 were expressed in podocyte precursors.

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