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LABORATORY STUDY

CD44 immunoreactivity in the developing human kidney: a marker of renal progenitor stem cells?

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Abstract

CD44 is a transmembrane adhesion glycoprotein, functioning as a hyaluronan receptor and participating in the uptake and degradation of hyaluronan. Recently, CD44 has been proposed in the adult kidney as a marker of activated glomerular parietal epithelial cells, the putative niche stem cells that, in case of damage to podocytes, might migrate inside the glomerular tuft and undergo transition to podocytes. Here, immunoreactivity for CD44 was tested in 18 human fetuses and newborns with a gestational age ranging from 11 to 39 weeks. CD44 immunoreactivity was observed in all but one developing kidneys, being localized in several renal cell types including intraglomerular, capsular, cortical and medullary interstitial cells and nerve cells. In some cases, CD44 marked scattered cells in nephrogenic subcapsular zone. Our data indicate that CD44 is involved in human nephrogenesis, probably marking a subset of progenitor/stem cells involved in early phases of kidney development and, putatively, in podocyte and/or interstitial cell differentiation.

Keywords

CD44, fetus, immunohistochemistry, kidney, mesenchymal cells, MET, nephrogenesis

History

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Introduction

CD44 is a transmembrane adhesion glycoprotein, which functions as a hyaluronan receptor and participates in the uptake and degradation of hyaluronan.[1,2] It is expressed in a wide variety of cell types, including parenchymal cells, macrophages and leukocytes, playing a crucial role in cell adhesion, migration and inflammation.[3] Moreover, CD44 has been shown to regulate hematopoietic progenitor distribution and granuloma formation.[4] A recent study evidenced an important dual role for CD44 in pneumococcal pneumonia in mice: it facilitates bacterial outgrowth and dissemination in the acute phase, but contributes to stop the inflammatory response in the resolution phase.[5] Recently, CD44 has been proposed in the adult kidney as a marker of activated glomerular parietal epithelial cells, the putative niche stem cells that, in case of damage to podocytes, might migrate inside the glomerular tuft and undergo transition to podocytes.[6]

To the best of our knowledge, no previous study investigated the role of CD44 during human kidney development, focusing on the expression and distribution pattern of this marker in different renal structures during kidney embryogenesis.[7,8] In this study, we sought to investigate CD44 immunoreactivity during nephrogenesis, in order to verify its expression pattern during mesenchymal to epithelial transition of metanephric mesenchymal cells, as well as its immunostaining in the different sequential structures typically involved in glomerulogenesis and tubulogenesis in the developing human kidney.

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Patients and methods

The expression of CD44 was evaluated in kidneys, from 18 human fetuses and newborns, ranging from 11 to 39 weeks of gestation. Clinical data of each case is reported in Table 1. In each fetus kidneys were obtain at autopsy and the major cause of death was asphyxia. In each subject, kidney samples were fixed in 10% buffered formalin, routinely processed, paraffin-embedded and 4 μ-thick sections for immunostaining were performed. Tissue sections were then dewaxed, rehydrated through graded alcohols and pre-treated for immunohistochemical analysis with 10 min heat-induced epitope retrieval in buffer pH 6.00 (EnVisionTM FLEX Target Retrieval Solution Low pH – Dako Denmark A/S, Glostrup, Denmark, Code: K8005). Slides were then incubated for 20 min at room temperature with anti CD44 (HCAM – Santa Cruz Biotechnology, Dallas, TX, Ync, code sc-7297) mouse monoclonal antibody clone DF1485 at 1:50 dilution. Staining procedures were performed by EnvisionTM FLEX+ (Dako Denmark A/S, Glostrup, Denmark, code: K8002) Detection System and AutostainerLink 48 instrument

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Table 1. Clinical data and gestational age.

No.	Gestational age (weeks)	Gender	Clinical data
1	11	Female	Voluntary interruption of pregnancy
2	19	Male	Acute chorioamnionitis
3	24	Male	Sepsis
4	26	Male	Asphyxia
5	26	Male	Asphyxia
6	26	Female	Asphyxia
7	26	Female	Asphyxia
8	26	Male	Asphyxia
9	27	Male	Asphyxia
10	27	Male	Asphyxia
11	27	Male	Asphyxia
12	27	Female	Asphyxia
13	28	Female	Asphyxia
14	30	Male	Asphyxia
15	31	Male	Asphyxia
16	35	Male	Asphyxia
17	38	Male	Neuroblastoma
18	39	Male	Pneumonia

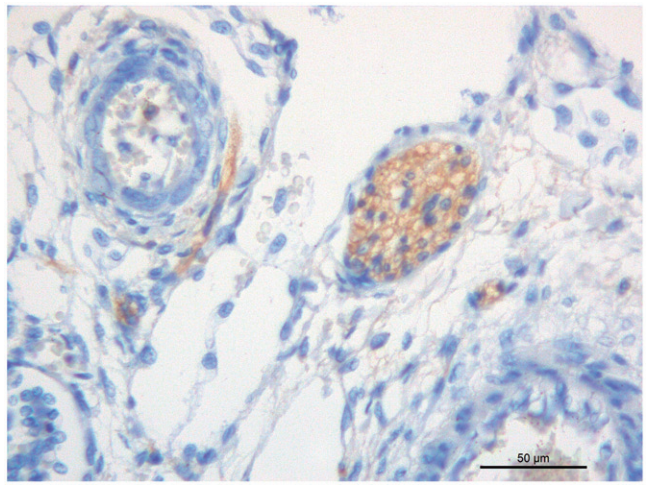

Figure 1. Case No. 3, 24 weeks, and original magnification 40x: nerve cells were immunostained by the anti-CD44 antibodies.

Table 2. CD44 immunohistochemical reactivity.

No.	Subcapsular nephrogenic region	Intraglomerular cells	Cortical interstitial cells	Nerves	Medullary interstitial cells	Capsula	Proximal tubules
1	–	–	–	+	–	–	–
2	+	+	+	+	–	+	–
3	–	–	+	+	–	–	–
4	–	–	–	–	–	–	–
5	–	+	+	+	–	–	–
6	–	+	+	+	–	–	–
7	+	+	+	+	–	–	–
8	+	+	+	+	–	–	–
9	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+
11	–	+	+	+	–	+	–
12	–	+	+	+	–	–	–
13	–	+	–	+	–	–	–
14	–	+	+	+	–	–	–
15	–	+	+	+	–	–	–
16	–	–	–	+	–	–	–
17	–	+	+	+	+	–	+
18	–	+	+	+	+	+	+

following dealer's instructions. Negative controls samples were incubated without mouse anti-CD44 polyclonal antibody. As positive control a sample of tonsil was used. All procedures performed were in accordance with the ethical national standards of the responsible committee on human experimentation.

Results

In this study, immunoreactivity for CD44 in renal cells showed some peculiar features. First of all, a marked interindividual variability was observed among fetal and neonatal kidneys, ranging from completely negative cases to some kidneys in which immunostaining for CD44 characterized the whole renal parenchyma. The interindividual variability in CD44 reactivity was not strictly bound to gestational age: in fact, marked differences were found even among newborns with the same gestational age (Table 2).

Differences among the fetal and newborn kidneys were also found regarding the cell type immunostained by CD44. Whereas nerve cells were immunostained by the anti-CD44 antibodies (Figure 1), the immunoreactivity of the other renal compartments and cell types changed from one case to the next. Capsular cells were immunostained in five out of 14 cases (Figure 2); intraglomerular cells were CD44-reactive in 12 out of 14 kidneys (Figure 3); scattered cortical interstitial stromal cells appeared CD44-positive in 12 cases (Figure 2); ductal cells showed immunoreactivity for CD44 in four developing kidneys (Figure 4); interstitial stromal cells of the medulla were CD44-positive in three cases (Figure 5) (see also Table 2).

Glomerular reactivity was mainly detected in few glomeruli, whereas the majority of glomerular bodies were negative. In the subset of glomeruli showing reactivity for CD44, one or two reactive cells were constantly detected inside the glomerular tuft, intermingled among the podocyte precursors (Figure 3). No reactivity was found in parietal cells of the Bowman capsule.

Interindividual differences were detected regarding the intensity of the immunostaining. In the vast majority of cases,

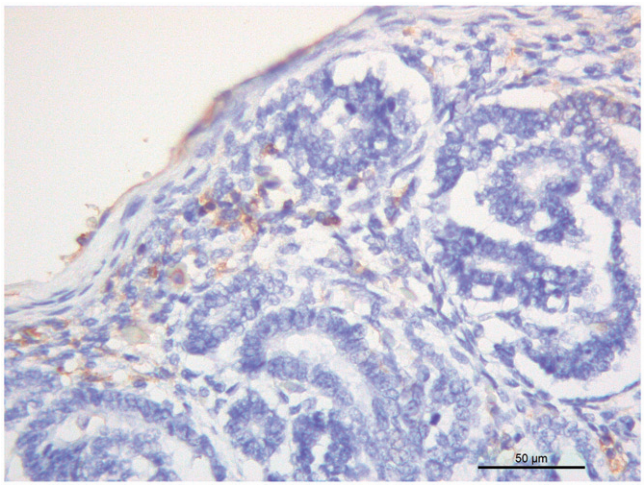

Figure 2. Case No. 12, 27 weeks, original magnification 40x: capsular cells and subcapsular cortical interstitial stromal cells immunostain.

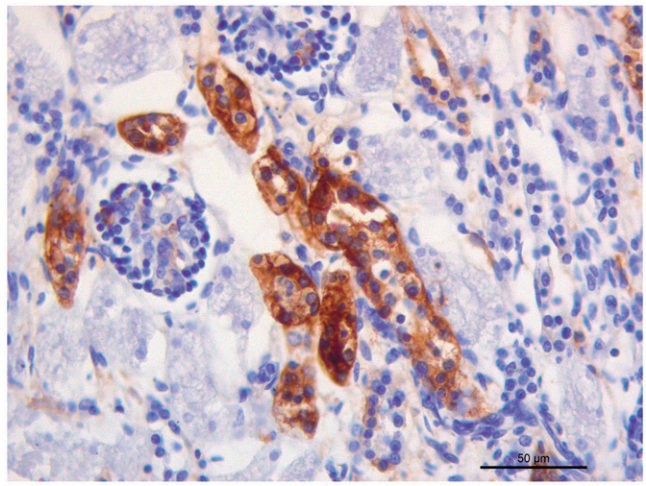

Figure 4. Case No. 17, 39 weeks, and original magnification 40x: proximal ductal cells immunoreactivity.

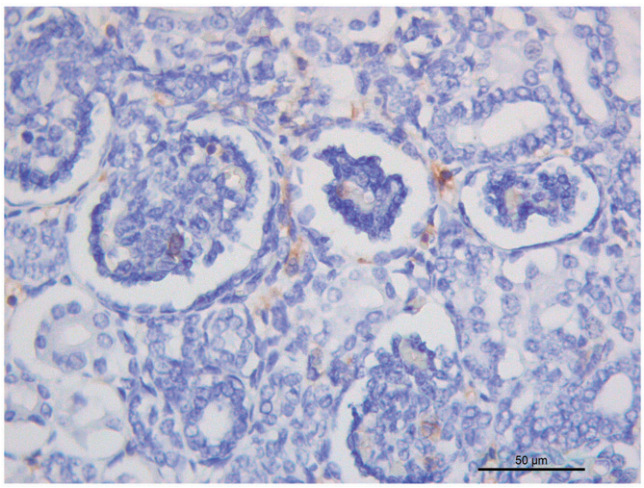

Figure 3. Case No. 12, 27 weeks, original magnification 40x: intraglomerular and cortical interstitial stromal cells immunostain.

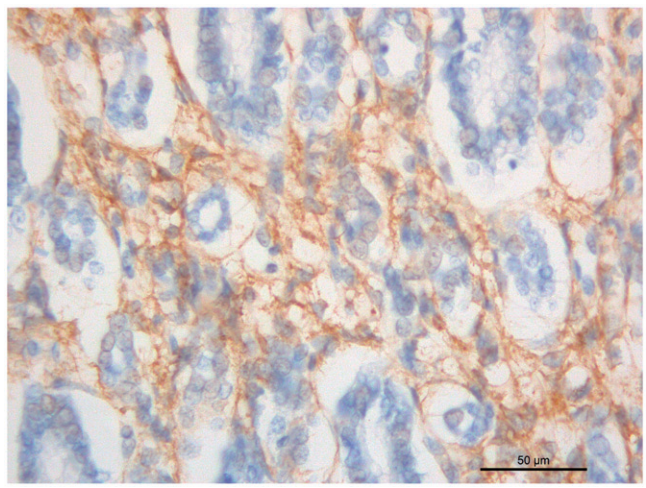

Figure 5. Case No. 10, 27 weeks, original magnification 40x: interstitial stromal cells of the medulla immunoreactivity.

immunostaining appeared weak, reaching the highest values in the nerve fibers that, according with our data, could be considered as an internal positive control. On the contrary, in few cases the reactivity for CD44 was very strong. In particular, we want to lay stress on cases No. 9 and No. 10, in which reactivity for CD44 in cortical and medullary stromal interstitial cells appeared very strong (Figure 5), and case No. 17, in which the protein was highly expressed in tubular cells (Figure 4).

Discussion

In previous studies focused on the identification of renal stem cells in adult kidneys,[9,10] CD44 expression has been associated with the activation of glomerular parietal epithelial cells (GPECs), a subset of cells lining the inner aspect of Bowman's capsule.[11] GPECs have been shown to migrate into the glomerular tuft and differentiate into mature podocytes.[12] Moreover, a subset of GEPCs in the Bowman's capsule of the adult human kidney show features of multipotent progenitor cells, and participate in renal repair.[13] CD44 immunoreactivity has been recently confirmed in an *in vitro* study in adult murine kidney GPECs. In that study, the acquisition of expression of CD44 was associated with the epithelial–mesenchymal transition (EMT) of GPECs ending with the acquisition of traits of renal progenitors.[14] Taken all together, these data indicate CD44 as a marker of activation of parietal epithelial cells of the glomeruli, possibly acting as potential stem cells able to repair the kidney after injury.[15]

Our data on CD44 expression in the developing human kidney add some new data to this picture. In fetal and neonatal kidneys here analyzed, CD44 was not restricted to glomerular cells, being detected in several other kidney cell compartments, including capsular, subcapsular, tubular and interstitial and nerve cells. Regarding immunoreactivity in glomeruli, we did not detect any significant reactivity for CD44 in cells of the Bowman's capsule: immunostaining for the glycoprotein was observed in isolated cells inside the glomerular tuft, in the absence of any reactivity in developing GPECs. Our data may suggest that, during development, CD44-reactive multipotent/stem cells have a different location as compared to the adult kidney, being predominantly detected in podocyte

location (Figure 3). We may speculate that, in the mature kidney, CD44-reactive stem/progenitor cells might migrate to the Bowman's capsule, GPECs becoming niche stem cells in adulthood. In focal segmental glomerular sclerosis, as well as in other podocytopathies, CD44-positive stem cells might migrate into the glomerular tuft, reaching the same position here observed during kidney development, originating new podocytes.[6]

Another new finding emerging from our study is that CD44 reactivity is not restricted to the nephron lineage, being detected in renal cells of the stromal lineage, including capsular and interstitial cells.[7] The ability of GPECS to de-differentiate into embryonic phenotype similar to that of myofibroblasts has been previously reported in renal pathology, in the evolution of glomerular crescents.[16] The detection, in this study, of a common CD44 immunoreactivity in glomerular and in interstitial cells in the developing kidney casts some doubt on the origin of renal interstitial cells, suggesting that at least a subset of them might originate from multipotent glomerular cells and not from a stromal progenitor.

The finding of a strong immunoreactivity for CD44 in tubular cells in one infant (Figure 4) affected by congenital neuroblastoma deserves some consideration. Tubular epithelial cells have been shown to respond to injury by de-differentiating into pluripotent/stem mesenchymal cells,[17] thus recapitulating the process active during nephrogenesis.[18] The strong immunostaining for CD44 of tubular cells in this infant might suggest that ductal cells during kidney development are able to de-differentiate toward an embryonic phenotype in same peculiar pathological conditions, here represented by the insurgence of neuroblastoma.

Another interesting finding of our study is represented by the marked interindividual variability in CD44 immunostaining of renal cells in the fetuses and newborns here analyzed. The intensity of reactivity for the glycoprotein was not associated with gestational age, or for body weight. Significant changes were also found regarding CD44 expression in the different renal cell compartments, some case showing a predominance of glomerular staining, others being characterized by a preferential interstitial immunoreactivity. The reason for this variability remains, at the best of our knowledge, unknown.

In conclusion, our study cearly shows the involvement of CD44 in human kidney development, evidencing its reactivity in a much larger number of cell types than previously reported. Further studies are needed, in order to better clarify the role of CD44-reactive multipotent cells in human kidney development, that might help to better understand the mechanisms at the basis of renal stem cell involvement in renal repair and regeneration[19] in childhood and adulthood kidney disease.

Declaration of interest

The authors report no conflict of interest.

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