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Review

Shift of galectin-3 expression in the human kidney during development

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Abstract

Galectin-3 (Gal-3) is a member of the lectin family, including 14 mammalian galectins, and has been shown to be involved in the many biological processes. In fact it has been reported to be expressed during human nephrogenesis, in the ureteric bud tips and in the medullary regions. In 11 developing human kidney the immunoexpression of Gal-3 was studied. Previously observations on Gal-3 expression in collecting ducts were confirmed and a wild variable reactivity was detected among the range from 20 to 36 weeks of gestational age considered. Between the early and late phases of gestation two phases have been identified: the first, from 20 up to 26 weeks of gestation, with a strong reactivity and the second, from 30 to 36 weeks, with a decrease in Gal-3 expression. This finding clearly indicates a major role for Gal-3 in early human nephrogenesis ending around the 30th week of gestation. In conclusion, Gal-3 apparently plays a role in kidney development at different check points, participating both to ureteric bud proliferation and to differentiation of structures originating from the metanephric mesenchyme.

Keywords

Galectin-3, nephrogenesis, newborn kidney, renal development, immunohistochemistry.

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Introduction

Galectin-3 (Gal-3) is a member of the lectin family, which includes 14 mammalian galectins [1]. Gal-3 is encoded by a single gene, LGALS3, located on chromosome 14, locus q21-q22 [2]. This protein has been shown to be involved in the following biological processes: cell adhesion, cell activation and chemoattraction, cell growth and differentiation, cell cycle, and apoptosis [3]. Given Gal-3's broad biological functionality, it has been demonstrated to be involved in cancer, inflammation and fibrosis [4], heart disease and cardiac dysfunction [5], and postischemic brain tissue remodelling following stroke [6]. Gal-3 has also been implicated in embryogenesis: it has been reported to be expressed during human nephrogenesis, in the ureteric bud tips and in the medullary regions [7].

The aim of the present study was to study Gal-3 immunoexpression in the developing human kidney at different stages of development, in order to better define its role in the kidney compartments during embryogenesis.

Patients and methods

The expression of Gal-3 was evaluated in kidneys, from 11 human fetuses, ranging from 20 to 36 weeks of gestational age. In each subject, we obtained, at autopsy, kidney samples, that were fixed in 10% buffered formalin, routinely processed and paraffin-embedded. Tissue sections were then dewaxed, rehydrated through graded alcohols and pre-treated with 20 minutes heat-induced epitope retrieval in buffer pH 6.00 (EnVision™ FLEX Target Retrieval Solution Low pH – Dako Denmark A/S, Glostrup, Denmark, Code: K8005). Immunohistochemical staining were performed using antibodies against Gal-3 (clone 9C4). Immunostaining was performed on 4 µ-thick sections; slides were incubated for 30 minutes at room temperature with a 1:50 dilution of the monoclonal anti Gal-3 primary antibody. Staining procedures were performed by Dako REAL™ EnVision™ Detection System Peroxidase (Dako Denmark A/S, Glostrup, Denmark) following the manufacturer's instructions. Negative controls samples were incubated without mouse anti-Gal-3 polyclonal antibody. All performed procedures were in accordance with the ethical national standards of the responsible committee on human experimentation.

Results

The renal expression for Gal-3 resulted wild variable among the different gestational ages considered in this study. At 20 weeks of gestational age a cytoplasmatic immunoreactivity for Gal-3 was observed in scattered cells of distal tubules (**Fig. 1**); while in the renal medullar parenchyma the reactivity for Gal-3 was restricted in collecting tubles (**Fig. 2**); a membrane positivity or nuclear and cytoplasmatic reactivity for Gal-3 in scattered basal cells was observed in the transitional epithelia lining the renal pelvis (**Fig. 3**). No immunostaining was found in glomeruli, and in the proximal tubules. Between 22 and 24 weeks of gestational age the reactivity for Gal-3 was very similar to what previously reported at 20 weeks of gestational age, but a strong nuclear and cytoplasmatic immunoreactivity for Gal-3 in distal tubules as the only difference (**Fig. 4**). At 26 weeks of gestational age a new pattern of immunoreativity for Gal-3 was found in collecting tubules, where instead of the diffuse reactivity detected in previous cases at 20 and between 22 and 24 weeks weeks of gestational age, only scattered cells were immunostained with a strong nuclear and cytoplasmic reactivity (**Fig. 5**). In the subcapsular zone, renal vesicles and S-shaped bodies showed a mild reactivity for Gal-3, mainly localized at the apical border. Gal-3 immunoreactivity cells were observed in the transitional epithelium of the renal pelvis (**Fig. 6**) with the same reactivity previously reported. At 30 weeks of gestational age a strong immunostaining for Gal-3 in the cortex was restricted to distal tubules, where scattered tubular cells exhibited a higher immunoreactivity, when compared to the surrounding cells. Gal-3 was also highly expressed in the medulla and in the

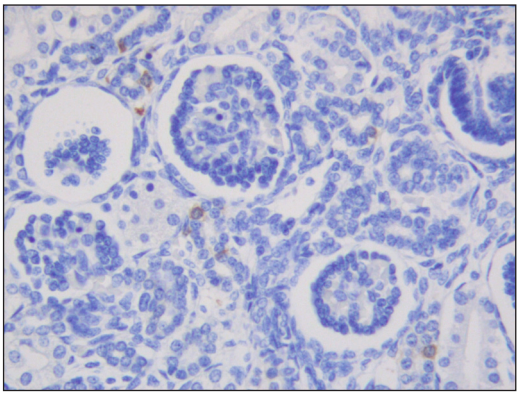

Figure 1. 20 weeks: immunoreactivity for Gal-3 in scattered cell distal tubules with cytoplasmatic reactivity.

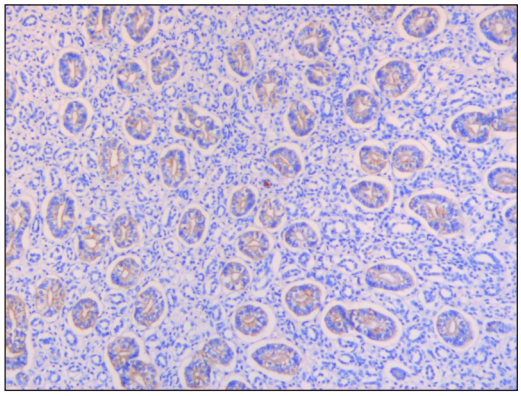

Figure 2. 20 weeks: reactivity for Gal-3 in to collecting tubles.

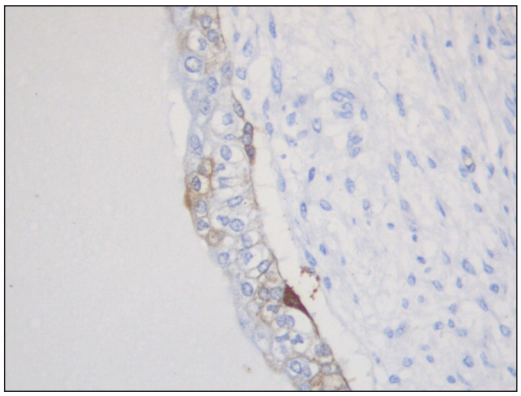

Figure 3. 20 weeks: membrane positivity for Gal-3 in the transitional epithelia or in basal scattered cell with nuclear and cytoplasmatic reactivity.

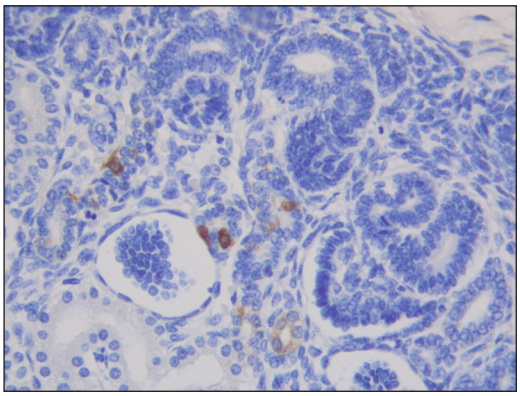

Figure 4. 24 weeks: strong nuclear and cytoplasmatic immunoreactivity for Gal-3 in distal tubules.

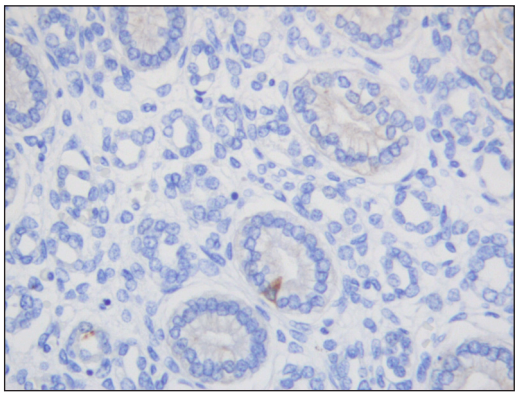

Figure 5. 26 weeks: only scattered cells were immunostained, with a strong nuclear and cytoplasmic reactivity.

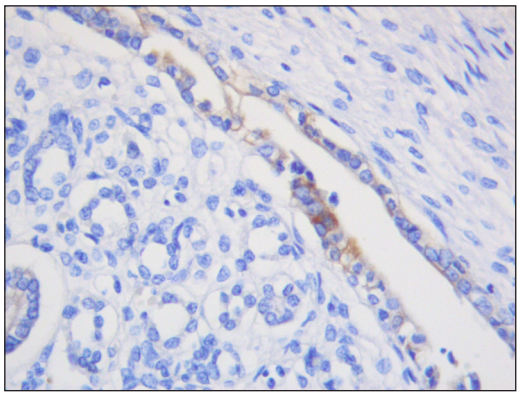

Figure 6. 26 weeks: Gal-3 immunoreactivity cells were observed in the transitional epithelium of the renal pelvis.

cytoplasm of collecting tubules. At 33 weeks of gestational age the immunostaining for Gal-3 was mild in all the renal compatments. In the cortex, Gal-3 was mainly detected in scattered epithelial cells of the distal tubules. Immunoreactivity in the medulla was very mild. Between 35 and 36 weeks of gestational age the reactivity for Gal-3 was apparently absent at low power of magnification, while at higher power, scattered immunoreactive cells were observed in distal tubules. No significant immunostaining was found in the other cortical structures, as well as in the medulla and in the transitional epithelium of the renal pelvis.

Discussion

The development of the human kidney is a complex process which requires interactions between pluripotential/stem cells, a part deriving from the metanephric mesenchyme and others originating from the ureteric bud emerging from the Wolffian duct. The interaction between these two progenitor cell types eventually leads to the coordinate development of multiple specialized epithelial, endothelial, mesangial and stromal cell types within the complex mature kidney architecture [8]. The histological analysis of the fetal human kidney allows researchers to be in the midst of a fascinating traffic of renal cells during different steps of their differentiation process: metanephric mesenchymal cells undergoing mesenchymal-epithelial transition; rudimental tubular structures, so called S-shaped bodies, transforming into glomeruli; collecting tubules, originating from the branching ureteric bud, fusing with distal tubules, originating from the S-shaped bodies; vascular and mesangial progenitors, derived from a multipotent metanephric mesenchymal cell, migrating into the scaffold of developing glomeruli, fusing with podocyte precursors [9]. Given this high number of cell types involved in human nephrogenesis, the interpretation of the developing human kidney, when exclusively based on morphology, represents an enigma difficult to be solved even in the hands of experienced pathologists and embryologists. The complexity of human nephrogenesis is well evidenced when cellular processes ongoing in human fetal kidneys are compared with those occurring in other animal species, often characterized by the utilization of different architectural and cellular ways during the early phases of glomerulogenesis [10].

A possible solution to reach a better interpretation of morphogenesis and of molecular mechanisms involved in human kidney development has been recently identified in the application of immunohistochemistry to renal embryology [11]. These immunohistochemical analyses were mainly aimed at defining the multiple stem/progenitor cell populations physiologically present in the nephrogenic zone located in close proximity to the renal capsule, recently defined "the subcapsular blue strip" due to the scarcity of cytoplasm of the scarcely differentiate renal cell precursors [12]. The aim of these studies was to characterize, by the detection of molecular markers, the stages of differentiation of the different renal cell types, in order to obtain a better comprehension of the degree of differentiation of a single kidney at birth, and look at old things such as the renal embryogenesis, with new eyes [8].

The vast majority of the immunohistochemical studies carried out on fetal kidney samples obtained at autopsy were focused on the identification of markers expressed by renal cells of the nephron lineage, including glomeruli, proximal, distal and collecting tubules [13]. The expression pattern of Wt1 [14], MUC1 [15, 16], Thymosin beta-10 [17], Thymosin beta-4 [18], CD10 [19] and CD44 [20] has been characterized, with new interesting data on the differentiation pattern of renal progenitors during the different phases of human nephrogenesis. Only few studies were focused on the non-nephron lineage, aiming at identifying molecular and immunohistochemical markers that might characterize cell types undergoing the interstitial cell fate, originating the cortical and medullary interstitium, the renal capsule, mesangium and pericytes in the developing kidney [21, 22].

In the present immunohistochemical study, we describe how Gal-3 is expressed in the human kidney in the different phases of human nephrogenesis. In a previous study, carried out on six fetal kidneys ranging from 17 up to 22 weeks of gestational age, immunoreactivity for Gal-3 was reported to be restricted to the ureteric bud and collecting tubules [7]. In our study, carried out on fetuses and newborns with gestational ages ranging from 20 up to 36 weeks, Gal-3 expression appeared much more complex, and characterized by marked differences between the early and late phases of gestation. As for the intensity of immunostaining, two phases have been identified: the first, including kidneys from 20 up to 26 weeks of gestation, is characterized by a strong immunoreactivity for Gal-3, diffuse to all the renal regions; the second, starting at 30 weeks and being unmodified till the 36th week, is characterized by a dramatic decrease in Gal-3 expression, decrease that contemporarily occurs in all kidney districts. This finding clearly indicates a previously unreported switch in Gal-3 expression in the human kidney, characterized by a major role for Gal-3 in early human nephrogenesis ending around the 30th week of gestation, and by a minor role for this protein in kidney development during the last weeks of gestation.

Intersting data emerge from our study concerning the immunolocalization of Gal-3 in the developing kidney. Our data confirm the observations by Winyard and coworkers on Gal-3 expression in collecting ducts [7]: moreover,

the finding of immunostaining for Gal-3 in the transitional epithelium of the renal pelvis, confirms its association with the structures developing from the ureteric bud. On the other hand, our data show that Gal-3 immunolocalization is not restricted to this component of human nephrogenesis: in fact, Gal-3 was highly expressed in the distal tubules, in the vast majority of cases (9/11), and occasionally in cells lining the proximal tubules. Since distal end proximal tubules derive from the metanephric mesenchyme [9], this study indicates that Gal-3 expression is not restricted to the ureteric bud and to the deriving structures, including collecting tubules and the renal pelvis, but shows that Gal-3 plays some role even in the structures originating from the metanephric mesenchyme through the cap mesenchyme, including distal and proximal tubules [8]. This hypothesis is confirmed by the finding, in three cases, of Gal-3 immunostaining in renal vesicles and in comma- and S-shaped bodies, the precursors of distal and proximal tubules.

Whereas Gal-3 presence in collecting ducts might be related to a role in the proliferation of the ureteric buds into the original metanephric mesenchyme, its detection in structures originating from the cap mesenchyme induces to hypothesize a role in the development of the proximal part of nephrons. In particular, on the basis of the high levels of Gal-3 being detected in the distal tubules, our data indicate a role for Gal-3 in the development of the distal part of the S-shaped body, the part originating the distal tubule and responsible for its fusion with the collecting tubules [9]. This hypothesis is reinforced by the finding, in this study, of no reactivity in glomeruli, originating from the proximal segment of the S-shaped body, and by the rarity of immunoreactivity in proximal tubules.

As for the intimate role of Gal-3 in the renal structures in which it is expressed during nephrogenesis, Gal-3 has been involved in cell adhesion, cell activation and chemoattraction, cell growth and apoptosis [3]. According with these data, we may speculate that Gal-3 expressed in the ureteric bud tips may play a relevant role in cell activation and chemoattraction toward the cap mesenchyme, i.e. toward the metanephric mesenchymal cells that condensate around the proliferating ureteric buds; Gal-3 expressed in renal vesicles could be involved in the development of mechanisms allowing previous mesenchymal cells to develop the cell machinery indispensable for cell adhesion; Gal-3 expressed in distal tubules might be involved in regulation of distal tubular cell growth.

In conclusion, our data lay stress on a new piece in the complex puzzle represented by human nephrogenesis: Gal-3, that apparently plays a role in kidney development at different check points, participating both to ureteric bud proliferation and to differentiation of structures originating from the metanephric mesenchyme. Further studies are needed in order to confirm our immunohistochemical data at molecular level.

Declaration of interest

The Authors declare that there is no conflict of interest.

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