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COMPARATIVE EXPRESSION OF PAX-2 AND OCT-4 IN FETAL, NORMAL ADULT, AND GLOMERULONEPHRITIC KIDNEYS

Abstract

Introduction: During organogenesis, the number and capacity of pluripotent stem cells capable of generating all types of kidney cells progressively decreases, and normal adult kidneys host a few immature multipotent cells with the capacity of self-regenerating.

Aims: We aimed to compare the expression of Pax-2, Oct-4, genes, which are responsible for the development and the differentiation of the embryonic stem cells in fetal, normal adult, and glomerulonephritic kidneys and compare these with CD133-main stem cell marker expression.

Material and Methods: Immunohistochemical analyses were performed with commercial antibodies against Pax-2, Oct-4 and CD133 on formalin-fixed, paraffin embedded tissue samples from 20 fetal kidneys with different gestational ages, 40 adult and 40 glomerulonephritic kidneys.

Results: The analyses showed a nuclear presence of both markers in fetal kidney structures, immature blastemic mesenchyme and early glomerular and tubular precursors, with decreasing to absent signals in imamture glomeruli, except in parietal cells. A weak Pax-2 signal was also present in the parietal cells and in some distal tubules of the adult normal

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kidneys, unlike Oct-4, which was entirely negative. An increased signal of both markers was observed in the glomerulonephritic kidneys: parietal glomerular cells and cellular crescents in cases of extra-capillary glomerulonephritis, and the number of positive atrophic tubules was higher ($p<0.05$) than the number of positive cortical tubules in normal adult kidneys. The interstitial cells in the three groups except rare cells were negative.

Conclusions: The presence of both markers in the immature fetal kidney points to the pluripotency of the mesenchymal-blastemic cells that precede the mature cells of all types, some of which undergo the process of mesenchymal-epithelial transition mediated by Pax-2. Their decreased expression in adult kidney tissue points to the differentiation and maturity of the cells that have lost their pluripotency. Since the hyperplastic crescent lesions are a result of the proliferation of parietal epithelial cells, Pax-2 and Oct-4 expression in cellular crescents indicates the presence of immature and undifferentiated cells which proliferate as a result of the impaired regeneration and differentiation of the damaged tissue. The positivity of the atrophic tubules can be attributed to the activation of the kidney's own potential stem cells in the process of defense and protection of its tissue.

Key words: Pax-2 and Oct-4, pluripotent kidney stem cells, glomerulonephritic lesions

Introduction

Traditionally, the kidneys have been considered to consist of stable cells with minimal capacity for regeneration. However, in the past decades investigations in this field have shown that the kidneys have a remarkable capacity for regeneration after damage has been done. This is due to the role of stem cells which are able to proliferate and differentiate in more than one terminally differentiated cell type (1, 2). Different authors have different ideas as to whether only immature structures might have progenitors capacity or mature differentiated tissues, in the conditions of injury might be activated and make use of the capacity of stem cells. Potential stem cells have been identified in the interstitium of normal adult kidneys (3) and at

the urinary pole of Bowmann's capsule (4) as well in fetal kidneys (5), and most of these had the characteristic expression of CD133 and CD24 (6). However, Oliver et al (2004) discovered that the renal papilla is a niche for adult kidney stem cells (9).

In our previous study on normal adult renal tissue and renal tissues with glomerulonephritic lesions we found the expression of CD133 in parietal epithelial cells. These were also found in rare proximal tubular epithelial cells and epithelial cells from the collecting ducts and Henle loop in cases from normal renal tissue as well as a higher expression of CD133 in the areas of the tubular lesions and interstitial inflammation.

It was postulated that the regeneration processes may recapitulate parts of the genetic program, which is evident during organogenesis, in order to reestablish proper tissue function after damage. During organogenesis, embryonal stem cells are the direct progenitors of kidney component cells, including podocytes, mesangial cells, and tubular epithelial cells, or via an additional appearance of kidney stem progenitor cells around mature kidney component cells. According to Saffirstain et al., third and fourth pathways are possible, including the role of extrarenal stem cells, either directly to mature kidney component cells or through the kidney stem progenitor cells to mature kidney component cells, including mesangial cells, podocytes and tubular epithelial cells.

Several genes have been shown to be modulated in response to kidney damage (7). Prominent among them is the expression of the immediate early genes that code for transcriptional factors that are rapidly and briefly expressed well before the onset of DNA synthesis. One of these genes is the transcription factor Pax-2, which is transiently expressed during nephrogenesis. This may be part of the genetic cascade leading to kidney regeneration in adulthood after kidney damage. It has been speculated that Pax-2 would show a biphasic expression pattern during kidney development and disease. M. Imgrund et al. (1999) in the study on experimentally induced ATN in adult mice, found transient temporally and locally restricted re-expression of Pax-2 in regenerating proximal tubular epithelial cells following kidney damage (8). During regeneration of the adult kidney, Pax-2 may play role by influencing proliferation.

Gupta et al. in her *in vitro* and *in vivo* differentiation of multipotent renal progenitor cells (MRPC-s) by ischemia-reperfusion experiment, found these cells expressed **vimentin**, **CD90**, **Pax-2** and **Oct-4,** but not cytokeratin, MHC class I and II and other markers of more differentiated cells. The authors proposed that MRPC participate in the regenerative response of the kidney to acute injury. **Oct-4** is required to prevent trophectodermal differentiation and, with the process of transcriptional repression, Oct-4, together with Nanog and Sox-2, are the key controllers of human embryonal stem cell pluripotency.

Data from the literature are mostly based on experimental animal models of embryonic tissues as well as in cell cultures. There are few data points for the studies done on human biopsy material, surgical, or autopsy material from fetal tissues.

The purpose of this study was to determine the expression of Pax-2 and Oct-4 genes responsible for the development and differentiation of embryonic stem cells in human fetal kidneys and to compare the results with the expression of Pax-2 and Oct-4 genes in in normal adult and glomerulonephritic kidneys. The final aim was to test the hypothesis that kidney stem cells exist in adult kidneys and that they participate in the processes of tissue reparation in damaged kidney tissue.

Material and Methods

The study included *100 cases divided in three groups:* (1) twenty fetal kidneys (FKs) received from autopsy archive material, (2) forty surgically extracted adult kidneys (Aks) due to diagnosed renal cell carcinoma, (3) forty renal biopsies taken in routine diagnostic procedures for glomerular disease (GD).

Tissue specimens from all cases from the three groups were fixed in 10% buffered formalin for 24 hours and paraffin embedded. Prepared paraffin sections were histochemically stained with H&E, PAS, PASM-Jones, and Trichrome Mason. Immunohistochemical analysis was done via the PT LINK immunoperoxidase technique for the following antibodies: Pax-2, Zymed, USA Polyclonal, 1:50, with nuclear staining in positive control cells

from Renal Cell Carcinoma; Oct-4, Santa Cruz, USA Polyclonal, 1:50, with nuclear staining in positive control cells in bone marrow, and CD133, Miltenyi, MoAC133, 1:11, with cytoplasmic staining in positive stem cells from bone marrow. The slides were then analyzed on a NICON Eclipse 2000 microscope, and the results were photographically documented.

The results have been statistically analyzed with Statistica 7.0, StatSoft Inc. software for descriptive and (non) parametric analyses.

Results

In the study of the first group, specimens from 20 fetal kidneys with a gestational age between 14th and 24th week were analyzed. In the second group, specimens from 40 surgically extracted adult kidneys (Aks) due to renal cell carcinoma, were analyzed. The mean age of the group was 59.4 years (minimum 34 years and maximum 79 years, and SD=8.4). In the third group, 40 renal biopsies taken in routine diagnostic procedures for glomerular disease were analyzed. The mean age of the group was 44.5years (minimum 21 years and maximum 73 years, SD=14.4).

Analysis for the Pax-2 expression in fetal kidneys revealed strong nuclear positivity in the S&comma shaped bodies from the 14th to the 24th gestational week, moderate positivity in the fetal glomeruli especially in the parietal epithelial cells in the 14th to the 16th gestational week and tubuli. In the medulla we found positivity in the cells from the collecting duct, Henle loop cells, and cells from the urothelial bud (Figure 1 A, B, C). Staining for Oct-4 in the fetal kidney specimens showed strong nuclear expression in the immature renal mesenchymal blastema, S&comma shaped bodies, parietal epithelial cells, tubuli and ureteral buds (Figure 1D). Immunohistochemical analysis for CD133 revealed positivity in the parietal cells of the immature glomeruli and rare cells in the proximal and distal tubuli, ureteral buds, and Henle loop cells. What is peculiar about this study is the finding of Oct-4 expression in rare podocytes of immature glomeruli and rare cells in the blood vessels of the fetal kidneys, as well as the low level of re-expression in GNKs.

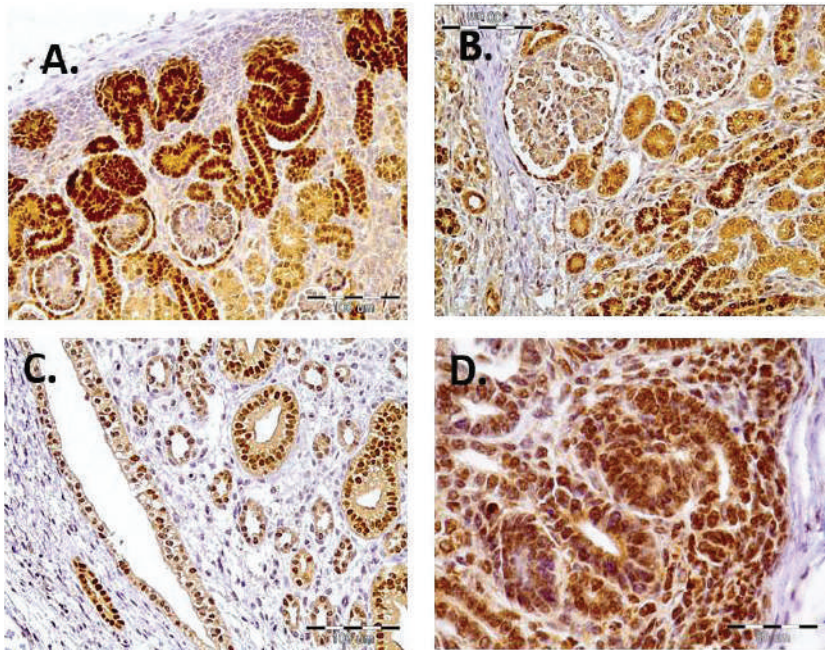

Figure 1 – (A) and (B) Pax-2 nuclear positivity in S & comma shaped bodies, positive parietal epithelial cells, and tubular epithelial cells; (C) Pax-2 positive epithelial cells in collecting ducts and urothelial bud; (D) Oct-4 nuclear positivity in renal mesenchymal blastema, S & comma shaped bodies and tubuli. (NIKON Eclipse 2000)

In the second group of adult mature kidney tissue, moderate Pax-2 positivity was found in the parietal cells of the glomeruli, collecting duct cells, and some of the distal tubules while Oct-4, was not present in any adult normal kidney structure (Figure 2A, B). Staining for CD133 revealed positivity in the parietal epithelial cells of the mature glomeruli and there was luminal positivity in the proximal tubuli and the cells of the Henle loop (Figure 2C, D and Table 1). In table 1 one can see that there is overlapping in the expression of CD133 with Pax-2 in parietal epithelial cells of the glomeruli, tubular epithelial cells, as well in the epithelial cells of the collecting duct.

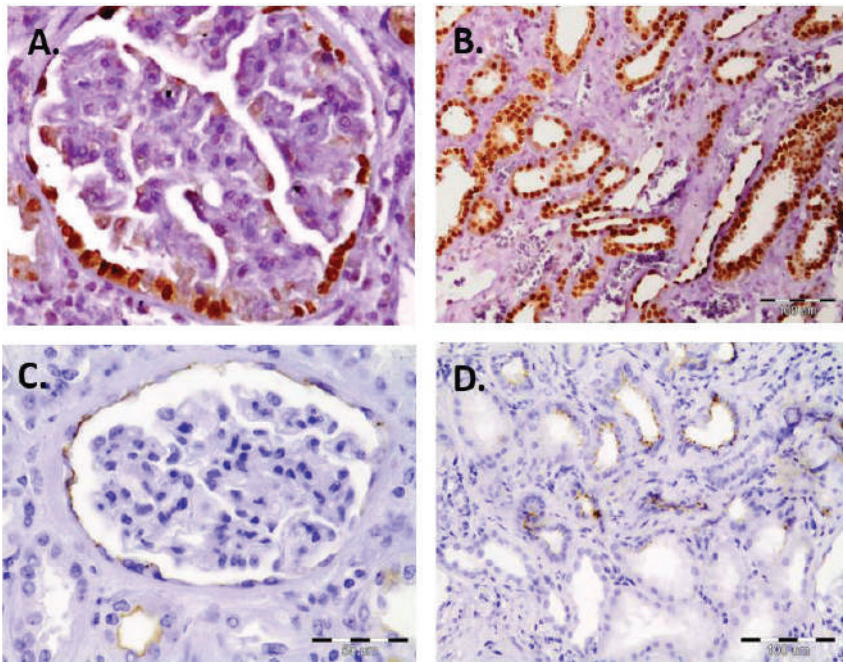

Figure 2 – (A) and (B) Pax-2 nuclear positivity in the parietal epithelial cells and epithelial cells of the collecting duct in adult kidney tissue; (C) and (D) CD133 expression in the parietal epithelial cells of mature glomeruli and tubular epithelial cells (NIKON Eclipse 2000).

Table 1

Expression of Pax-2 and Oct-4 in adult kidneys in relation with CD133 expression

	Expression of Pax-2 and Oct-4 in AKs Cortex			AKs Medulla
	Glomeruli	Atrophic tubules	Interstitium	
CD133	Parietal cells	Proximal	/	• Collecting • Henle loop
Pax-2	Parietal cells	Distal	/	• Collecting ducts
Oct-4	/	/	/	/

The third group consisted of tissues from 20 cases with diagnosed primary glomerular disease. Ten cases (25%) had mesangial glomeruloneph-

ritis, 9 cases (22,5%) had membranous glomerulopathy, 7 cases (17%) had IgA nephropathy, 8 cases (20%) had extracapillary glomerulonephritis, 4 cases (10%) had focal segmental glomerulosclerosis, and 2 cases (5%) had mesangioproliferative glomerulonephritis. The analysis of the results revealed a nuclear expression of Pax-2 similar to the expression in normal kidney specimens, i.e. expression in the parietal epithelial cells of the Bowman capsule, which was especially strong in the epithelial crescents of the extracapillary glomerulonephritis (Figure 3A). We also found positive expression in the areas of atrophic tubuli. Interestingly, the number of positive atrophic tubules was higher ($p<0.05$) than the number of positive cortical tubules in normal adult kidneys (Figure 3B). Higher level of expression has been found in the epithelial cells of collecting ducts. The interstitial cells were negative.

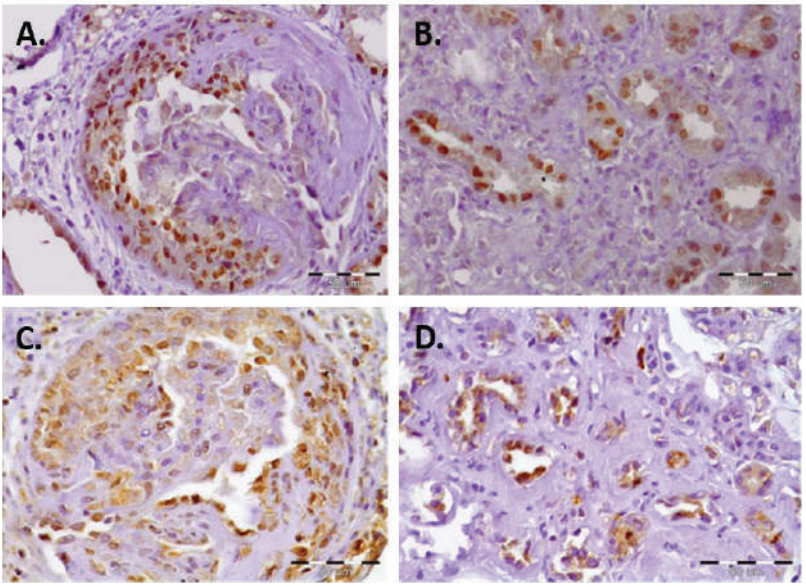

Figure 3 – (A) Pax-2 nuclear positivity in parietal epithelial crescents; (B) Pax-2 nuclear positivity in atrophic tubular epithelial cells; (C) Oct-4 nuclear positivity in parietal epithelial crescents (D) Oct-4 nuclear expression in atrophic tubular epithelial cells. (NIKON Eclipse 2000)

Cases with extracapillary glomerulonephritis and focal segmental glomerulosclerosis showed the strongest expression compared to other forms of glomerular lesions ($p<0.05$). This was confirmed via a strong correlation between the Pax-2 expression in tubular epithelial cells and interstitial fibrosis (Figure 4A). When we compared the level of expression in different forms of glomerular lesions and the control group, we found a significantly higher expression of Pax-2 positive tubuli between each of the entities and the control group (Figure 4B). One could speculate that this might be on account of a more extensive degree of damage of the glomeruli and the tubulointerstitial tissue in these forms of glomerulonephritic lesions.

Similar results were obtained when staining the specimens with Oct-4 (Figure 3C, D). We found a strong expression of Oct-4 in the epithelial crescents of the extracapillary glomerulonephritis, atrophic tubuli, and a small number of collecting duct cells. We also found low level of re-expression in glomerulonephritic kidneys in this group.

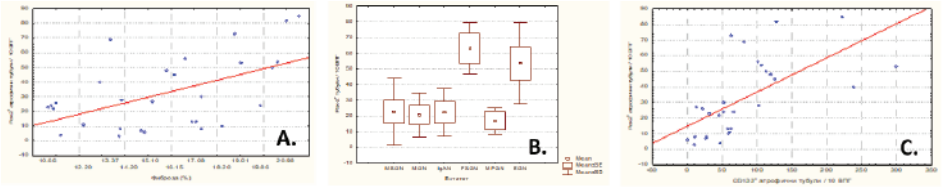

Figure 4 – (A) and (B) Pax-2 nuclear positivity in S & comma shaped bodies, positive parietal epithelial cells and tubular epithelial cells; (C) Pax-2 positive epithelial cells in collecting ducts and urothelial bud; (D) Oct-4 nuclear positivity in renal mesenchymal blastema, S & comma shaped bodies and tubuli.

CD133 expression in the glomerulonephritic group was found to be increased in the tubular epithelial cells in glomerulonephritic kidneys. Rare interstitial cells were also positive for CD133. We found a positive correlation between the Pax-2 and CD133 expression in the areas of atrophic tubuli (Figure 4C).

Table 2 displays the results from the comparative expression of Pax-2 and Oct-4 in the cortical and medullary tissue of glomerulonephritic lesions in relation to the expression of CD133 as the main stem cell marker. There is a strong overlap in the expression of Pax-2 and Oct-4 in the glomeruli and tubular compartments with partial overlapping with CD133 expression.

Table 2

Comparative expression of Pax-2 and Oct-4 in the cortical and medullary tissue of glomerulonephritic lesions in relation to the expression of CD133 as main stem cell marker.

	Comparative expression of Pax-2 and Oct4 in GNK - Cortex			Medulla
	Glomeruli	Tubules	Interstitium	
CD133	Parietal cells / Negative crescents	+++ (apico-lateral)	+	• Collecting ducts • Henle loop
Pax2	Parietal cells / Positive crescents	+++ (nuclear)	/	• Collecting ducts
Oct-4	Parietal cells / Positive crescents	+++ (nuclear)	/	• Collecting ducts

Discussion

We have presented a study on the expression of two transcriptional genes, Pax-2 and Oct-4, in kidney tissue from glomerulonephritic lesions and compared these with the expression of such genes in fetal kidneys and adult mature kidney tissue, having normal control groups of different ages. These results were compared with the expression of CD133, since it has been demonstrated the presence of CD133+ cells in a normal adult human kidney, which expressed Pax-2, is otherwise defined as an embryonic renal marker, suggesting their renal origin (3, 4, 5). These cells may respond to local environmental stimulation, with differentiation into endothelial or epithelial tubular cells, both *in vitro* and *in vivo*. The discovery of pluri-potent bone marrow-derived stem cells has raised the possibility that the stemness of the kidney tissue could be due to bone marrow derived stem cells, and there is a need to re-examine the cellular source further in the future (1, 5, 6). In our study, we showed the presence of cells that express activity of two immature transcriptional genes in both localizations – parietal epithelial cells as well in the cells of collecting ducts and ureteral epithelium. These results allow for the presence of at least two localizations in the kidney for cells with stemness capability – glomeruli and renal papilla.

During early embryogenesis, Oct-4 is a master transcriptional regulatory gene, and the presence of both markers in the immature fetal kidney structures points to the pluripotency of the mesenchymal blastemic cells that precede the mature cells of all types. Some of these cells undergo the process of mesenchymal-epithelial transition mediated by Pax-2. Their decreesed expression in adult kidney tissue points to the differentiation and maturity of the cells that have lost their pluripotency. Since hyperplastic crescent lesions are the result of proliferation of parietal epithelial cells, the presence of Pax-2 and Oct-4 in cellular crescents indicates that they are composed of immature and undifferentiated cells that proliferate as a result of impaired regeneration and differentiation of the damaged parietal epithelial cells. Another optional hypothesis is that the damaged parietal epithelial cells underwent a process of dedifferentiation with re-expression of immature transcriptional genes Pax-2 and Oct-2. The positivity of the atrophic tubules can also be attributed to the activation of the kidney's own resources, potential stem cells in the process of defense, and protection of its tissue. This ability of re-expression of immature transcriptional genes in the parietal epithelial cells, as well in some of tubular epithelial cells of damaged renal tissue, suggests their potential in the treatment of kidney damage.

Conclusions

Re-expression of Pax-2 and Oct-4 in the hyperplastic crescent lesions, which are result of proliferation of parietal epithelial cells, indicates that they are composed of immature and undifferentiated cells that proliferate as a result of impaired regeneration and differentiation of the damaged parietal epithelial cells.

The positivity of the atrophic tubules can also be attributed to the activation of the kidney's own resources, potential stem cells in the process of defense, and protection of its tissue in the process of epithelial mesenchymal transformation.

The findings of Oct-4 expression in rare podocytes of immature glomeruli and rare cells in the blood vessels of the fetal kidneys, as well as its low level of re-expression in GNKs displays that some bone marrow stem cells might also play a role in the process of regeneration of damaged kidney tissue.

Disclaimer: The authors declare that the research was conducted in the absence of any commercial or financial relationship such that the research cannot be constructed as a potential conflict of interest.

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КОМПАРАТИВНА ЕКСПРЕСИЈА НА PAX-2 И OCT-4 КАЈ ФЕТАЛНИ, НОРМАЛНИ ЗРЕЛИ И ГЛОМЕРУЛОНЕФРИТИЧНИ БУБРЕЗИ

Абстракт

За време на органогенезата, бројот и капацитетот на плурипотентите матични клетки способни да ги продуцираат сите видови на клетки прогресивно се намалува, и нормалните зрели бубрези содржат малку незрели мултипотентни клетки со капацитет за само-регенерација.

Нашата цел беше да се спореди експресијата на Pax-2 и Oct-4, гени кои се одговорни за развојот и диференцијацијата на ембрионалните матични клетки кај фетални, нормални зрели и гломерулонефритични бубрези и да се споредат со експресијата на главниот маркер на матични клетки CD133.

Ние направивме имунохистохемиска анализа со комерцијални антитела за Pax-2, Oct-4 и CD133 на формалин фиксирани и парафин вклопени ткивни примероци од 20 фетални бубрези со различна гестациска старост, 40 зрели и 40 гломерулонефритични бубрези.

Анализата покажа јадрено присуство на двата маркери во феталните бубрежни структури, незрелиот бластемски мезенхим и раните гломеруларни и тубуларни прекурзори, со намалување до отсуство на сигналот во незрели гломерули, освен во париеталните клетки. Слаб Pax-2 сигнал беше присутен исто така во париеталните клетки и во некои дистални тубули на зрели нормални бубрези, за разлика од Oct-4, кој беше негативен во целост. Зголемен сигнал за двата маркери беше најден кај гломерулонефритични бубрези: париетални гломеруларни клетки и клеточни кресценци и во случаите со екстракапиларен гломерулонефритис, а бројот на позитивни атрофични тубули беше повисок ($p<0,05$) од бројот на позитивните кортикални тубули во нормалните зрели бубрези. Интерстицијалните клетки во трите групи освен ретки клетки беа негативни.

Присуството на двата маркери во иматурни фетални бубрези укажува на плурипотентност на мезенхималните-бластемски клетки кои претходат на зрелите клетки од сите видови, од кои некои подлежат на процес на мезенхимална-епителна транзиција посредувана со Pax-2. Нивната намалена експресија кај матурните зрели бубрези укажува на диференцијација и зреење на клетките кои ја изгубиле нивната плурипотентност. Бидејќи хиперпластичните кресцентни лезии се резултат на пролиферација на париеталните епителни клетки, Pax-2 и Oct-4 експресијата во клеточните кресценци индицира присуство на иматурни и недиференцирани клетки кои пролифериираат како резултат на оштетена регенерација и диференцијација на оштетеното ткиво. Позитивноста на атрофичните тубули може да се припише на активација на бубрежните сопствени потенцијални матични клетки во процесот на одбрана и заштита на ткивото.

Клучни зборови: Pax-2 и Oct-4, плурипотентни бубрежни матични клетки, гломерулонефритични лезии