IJKD KIDNEY DISEASES

Embryohistiogenesis of Vascular Tufts of Glomeruli
A Possible Hypothesis

Shahriar Dabiri, Mohammad Mehdi Moeini-Aghtaei, Bahram Dabiri

Pathology and Stem Cell Research Center, Department of Pathology, Afzalipour Hospital, Kerman University of Medical Sciences, Kerman, Iran

Keywords. embryology, kidney, glomeruli, vascular tuft

Introduction. Embryogenesis of the kidney glomeruli, especially its vascular component, has not been well documented. Glomeruli capillary tuft is surrounded and enveloped by visceral epithelial cells, which is a unique portal system that connects afferent with efferent arteriole without interaction with venular circulation. We hypothesized that the portal system embryologically has developed by extension of the intima of afferent arteriole into the stroma of glomerulus. We also hypothesized that juxtaglomeruli apparatus was developed from remnants of smooth muscle cells of the media of afferent arteriole at the anastomosing site with the Bowman capsule entrance.

Materials and Methods. We studied 5 human fetal kidneys by hematoxylin-eosin, periodic acid-Schiff, and immunoperoxidase staining techniques.

Results. Hematoxylin-eosin staining of fetal kidney showed presence of erythrocytes in early vesicle form of glomeruli that was confirmed by immunohistochemical staining with CD31, smooth muscle actin, and CD34 markers. These stains showed extension of extraglomerular arterioles to the glomeruli. Periodic acid-Schiff staining showed also the continuity of the basement membrane in extraglomeruli and internal glomerular vascular tufts.

Conclusions. This study shows that there is a relationship between the metanephric blast cells and major vessel critical for angiogenesis. When afferent arteriole come in contact with the immature glomeruli, its intima migrates into the glomerular tuft to form intraglomerular capillary system, while its smooth muscle remains at the entrance orifice and develops juxtaglomerular apparatus cells.

IJKD 2017;11:360-6
www.ijkd.org

Original Paper

INTRODUCTION

The kidneys are extremely vascularized organs that receive about 20% to 25% of cardiac output critical to their function as an excretory organ.[1,2] Kidney function therefore depends on the development of a rich vascular network supplying blood to each nephron. Blood enters to each glomerulus from the afferent arteriole and exits through the efferent arteriole. In this process, the kidney is able to adjust its blood flow, filtration rate, acid-base balance, and clearance of waste material from blood as well as several other vital functions.[3] Studies have shown that in primary glomerular diseases, such as focal segmental glomerulosclerosis, minimal change disease, anti-glomerular basement membrane disease, and systemic vasculometabolic diseases, the pathologic process included abnormalities in glomerular capillary tufts.[4] The embryogenesis of glomerular capillary tuft and its connection with podocyte and mesangial cells, however, remains poorly understood.

Given the critical role that glomerular capillary tufts play in health and disease, we believe a better understanding of its embryogenesis could help explain some challenges we face in understanding pathophysiology of kidney disease.[5,6] Although there are new progresses in transplantation survival, many unsolved problems still remain with kidney allografts.[7] Better understanding of glomerular formation may also help investigators develop new methods in this area. In this study, we attempt to describe glomerular formation in kidney based on embryogenesis.

Normal glomerular structure within the Bowman capsule surrounded by mesangial cells and visceral epithelial cell is essential for normal filtration of plasma and formation of urine. Nephrons develop through maturation and differentiation of mesenchymal cells of mesonephric buds (the ureter, renal pelvis, major and minor calyxes, and collecting ducts) and metanephric blast cells (the Bowman capsule, proximal convoluted tubules, loops of Henle, and distal convoluted tubules). This is followed by development of immature compressed compact glomeruli and formation of mesangial cells in the tuft surrounded by visceral epithelial cells.[3,8] Lacunar space between visceral and parietal cells becomes the open space of the Bowman capsule.

We hypothesized that capillary loops originate from extension and elongation of intima of afferent arterioles into the tuft of mesangiovisceral epithelial cells of metanephric blast cells. Metanephric blast cells guide this loop migration between the cells of the tuft of mesangial and visceral epithelial cells by releasing vascular growth factor. The afferent arteriole leaves its remnant of smooth muscle at the site of overgrowth of intima rest on the orifice of transfer zone, and this is transformed into the juxtaglomerular apparatus close to the distal convoluted cells that are destined to form macula densa cells. To evaluate this hypothesis, we used immunohistochemistry methods utilizing different markers for glomerular including vascular components to define the relationship between intraglomerular and extraglomerular vessels.

MATERIAL AND METHODS

Kidney Tissue

We studied 5 human fetuses from the Department of Obstetrics and Gynecology of Afzalipour Hospital, Kerman Medical Sciences University. Fetal kidneys were extracted and first evaluated for their structural integrity and then fixed in 10% buffered formalin and paraffin-embedded for routine processing. Five-micron-thick paraffin sections were prepared for hematoxylin-eosin and periodic acid-Schiff staining.

Immunoperoxidase Staining

Antibodies used in this study are listed in Table 1. The 3-μm paraffin sections were deparaffinized in xylol and rehydrated by dilution series of alcohol. The sections washed by distilled water and unstained sections were steamed for 20 minutes for heat-induced antigen retrieval. The sections were then placed in 3% oxygen peroxide in methanol solution for 10 minutes to eliminate endogenous peroxidase. The sections were incubated with the mouse monoclonal antibodies for CD34 (Dako, QBEND 10 clone), smooth muscle actin (Dako, 1A4 clone), vimentin (Dako, Vg clone), polycolonal antibodies for CD117 (Dako, A4502 clone), and factor VIII (Dako, A0082 clone) diluent for 60 minutes in room temperature. The slides were washed with tris solution and then were incubated with antimouse/rabbit biotinylated bridging antibodies for 30 minutes. Finally, hematoxylin was used as a counterstainer.

RESULTS

The hematoxylin-eosin staining of 20-week kidney slides showed mostly mature glomeruli and nephron in areas near medulla in comparison with those in the cortical region. We saw nephron indifferent phase of maturation from vesicle to mature nephron (Figure 1). Hematoxylin-eosin slides were observed for capillary loop differentiation in mature glomeruli; capillary loops were prominent and they were connected to the large arterioles, which were located in extra spaces of glomeruli. Interestingly, we could find presence of erythrocyte between these cells in immature tuft of the glomeruli, which suggested that it might have originated from vessels in the primary tuft of the glomeruli (Figures 2 and 3).

The periodic acid-Schiff staining showed that basement membrane in the glomeruli were in the continuity of the extraglomeruli arterioles (Figure 4). In the entrance of glomeruli in the space between afferent arteriole and distal tubule, a collection

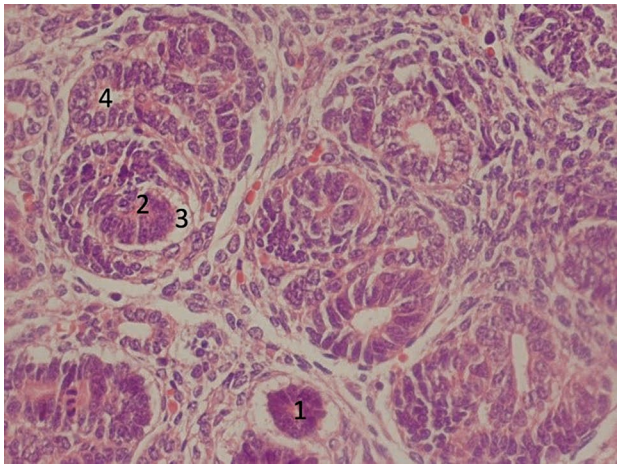

Figure 1. Immature glomeruli: vesicle (1), S-shaped glomerulus (2), the Bowman capsule (3), and the proximal tubule (4) (hematoxylin-eosin, × 400).

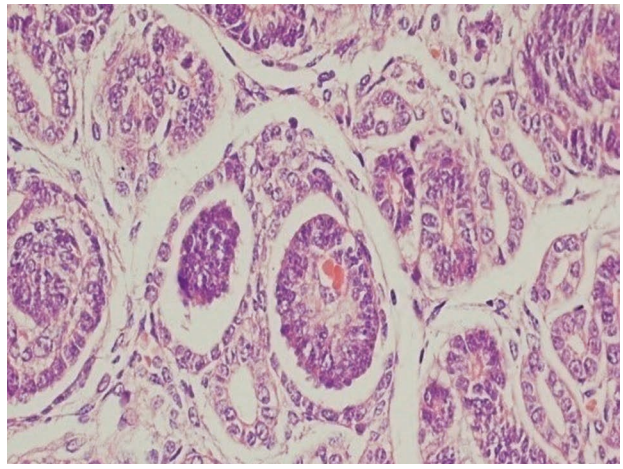

Figure 3. Vesicle form glomeruli containing a vessel with erythrocyte in it (hematoxylin-eosin, × 400).

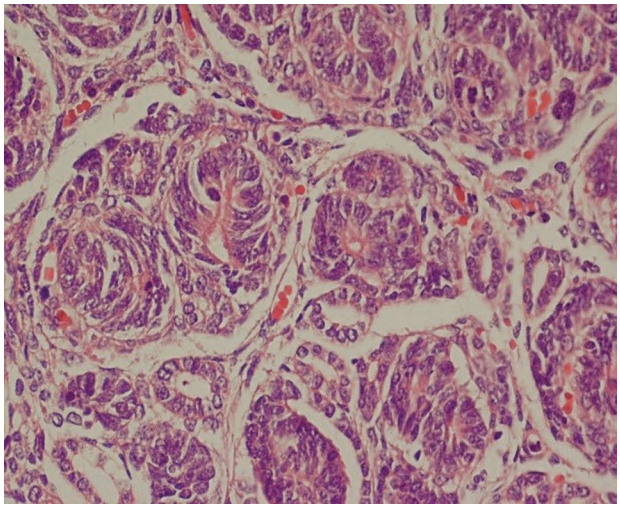

Figure 2. Developing glomeruli were surrounded by the extraglomerular vessels (hematoxylin-eosin, × 400).

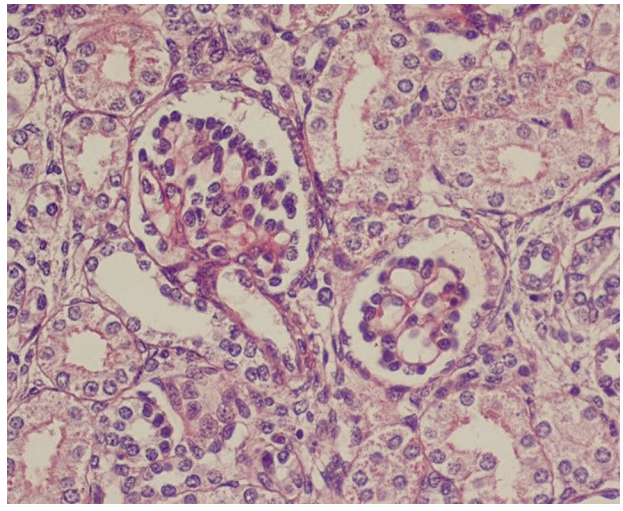

Figure 4. Continuity of intra- and extraglomerular basement membrane (periodic acid-Schiff, × 400).

of cells was observed which had stained more by periodic acid-Schiff (Figure 5). Periodic acid-Schiff staining weakly stained immature glomeruli.

The immunostaining for smooth muscle actin staining of capillary loops in the glomeruli showed that in some places, the capillary loops were in alignment with the stained extraglomerular arterioles (Figures 6 to 8). The CD34 confirmed the same pattern which showed that arteriole in the extraglomerular space continued to the glomerular space (Figure 9). The von Willebrand factor (factor VIII) stained vessel wall and spaces around glomeruli, proximal and distal tubules, and stroma (Figure 10). In this staining, we observed a collection of spindle cells near the distal tubule that might be the origins of the juxtaglomerular apparatus cells (Figure 11). Only immature glomeruli in the slides were weakly stained for CD117 (Figure 12). Staining for vimentin showed diffuse staining of the glomeruli and interstitial tissue (Figure 13).

A depiction of the study's hypothesis is shown in Figure 14.

DISCUSSION

Renal vascular development is one of the most controversial areas of kidney organogenesis.[2,3,9,10] This is especially true when we consider the process involved in construction of vasculatures.[11] In this study, we investigated the relationship between intraglomerular vessels and extraglomerular arterioles and used morphological and

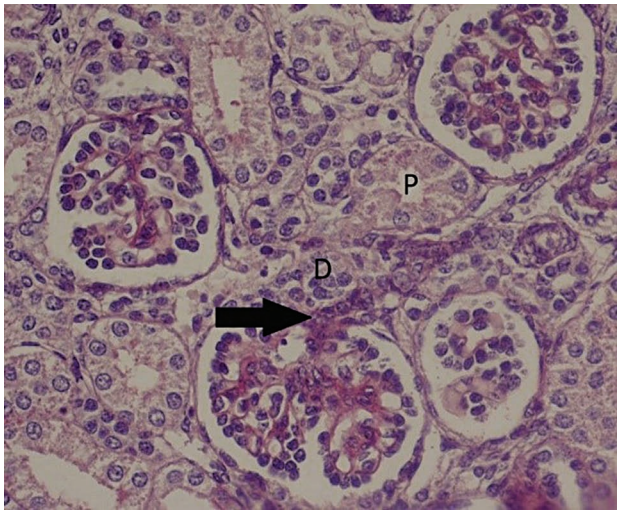

Figure 5. Collection of cells that stained stronger than adjacent cells (arrow) which be might be future juxtaglomerular apparatus cells, in touch with distal tubules cells (D) and proximal tubule (P) (periodic acid-Schiff, × 400).

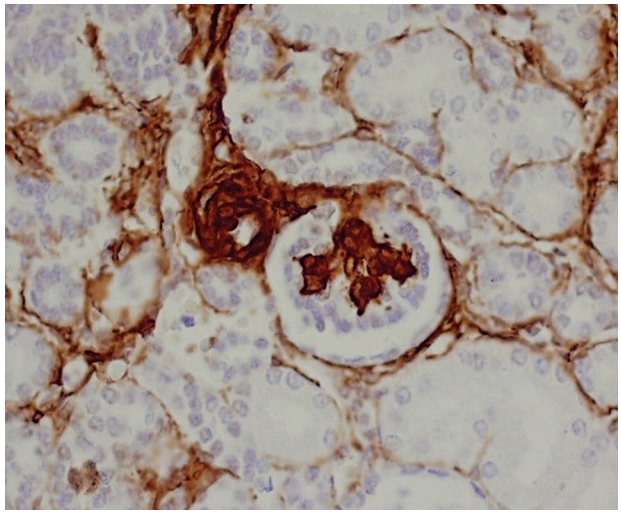

Figure 7. Continuity with arteriole of glomeruli vascular tuft (smooth muscle actin × 400).

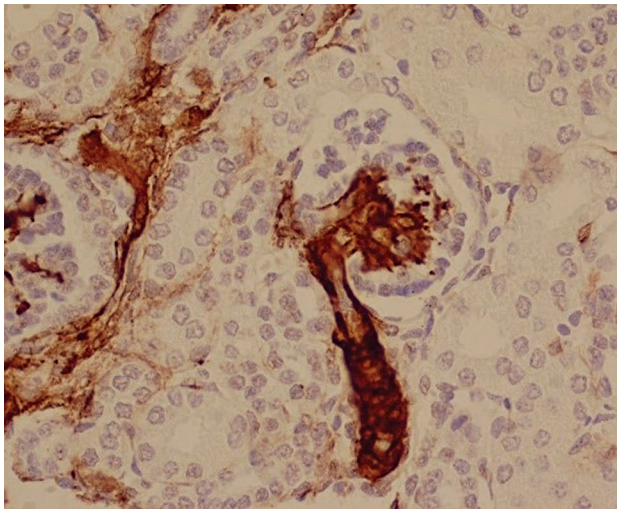

Figure 6. Immunostaining appeared mainly expressed in the extraglomerular arteriole and it seemed expressed in the afferent arteriole and its branches (smooth muscle actin × 400).

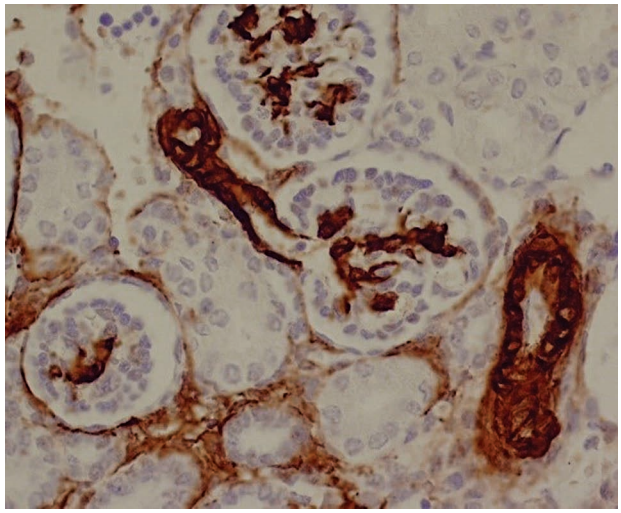

Figure 8. Staining was expressed in the afferent arteriole, and its connection with extraglomerular arteriole was shown (smooth muscle actin × 400).

immunohistochemical techniques.

Hematoxylin-eosin staining of 20 weeks fetal kidneys showed glomeruli in different phases of maturation. Sections showed metanephric cap and its connection to the ureteric bud. There are many arterioles in the stroma, with some of the arteriole entering glomeruli in different stages of maturation. Some sections showed erythrocytes in vesicle form of glomeruli, indicating that capillary tuft may enter the glomeruli before the mesenchymal-epithelial differentiation forming components of the Bowmen capsule. Morphologically, these sections showed that endothelial cells enter the glomeruli before metanephric cells maturation. The immunohistochemical staining for special endothelial makers showed that there was a strong correlation between endothelial cells in the glomeruli and extraglomerular vessels. It showed primitive endothelial cells in the immature metanephric blast cells were more mature than the latter cells. For this reason, we would suggest that these cells were endothelial cells of afferent arteriole.

This theory was demonstrated by immonohistochemical staining for the capillary markers such as CD34, smooth muscle actin, and factor

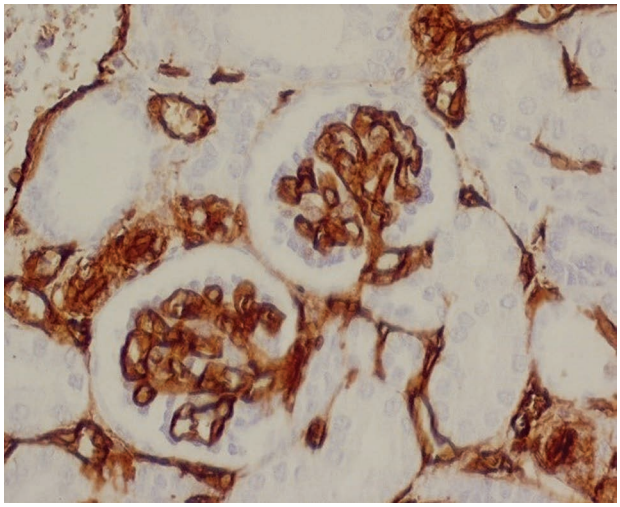

Figure 9. CD34 immunostaining showed afferent arteriole and its correlation with extraglomerular vessels (CD34, × 400).

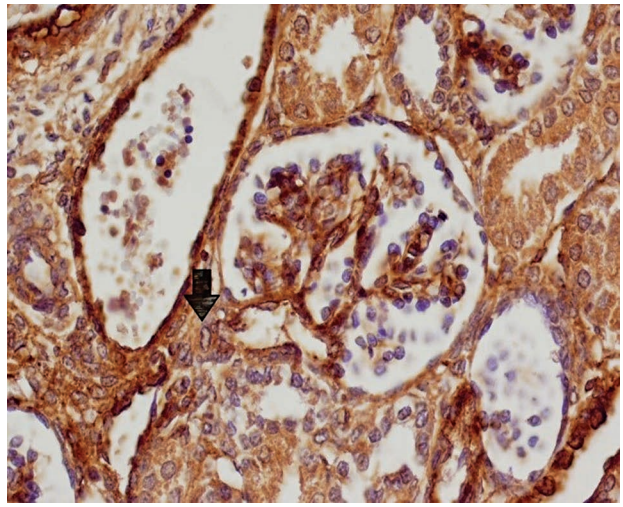

Figure 11. Factor VIII staining showed a collection of spindle cells (arrow) that might be a precursor of juxtaglomerular apparatus cells (factor VIII, × 400).

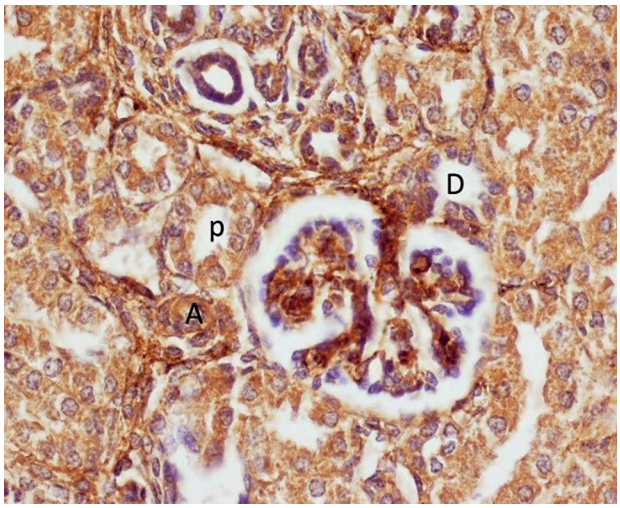

Figure 10. Factor VIII expression showed arteriole (A), distal tubule (D), and proximal tubule (P) (factor VIII, × 400).

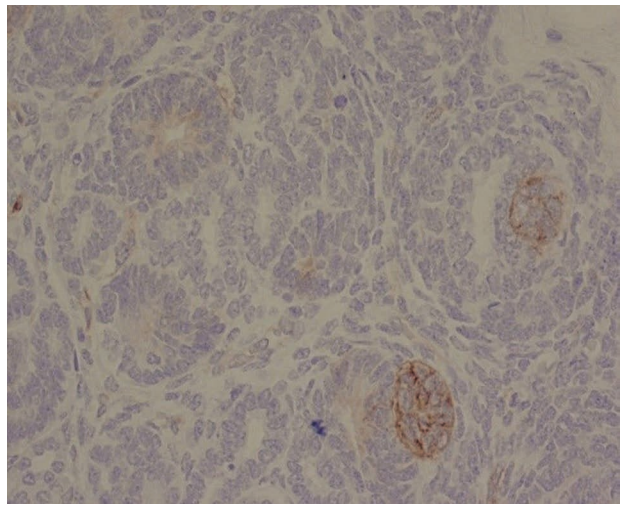

Figure 12. CD117 was expressed in immature glomeruli (CD117, × 400).

VIII. The staining showed the correlation between intra- and extra-arterioles differentiations of the Bowman capsule. To emphasis the formation of intimal loop extension of afferent arterioles, we compared the previous researches works focusing of origin of this complicated vasculature tufts of the glomeruli with our findings.

Lopez and colleagues suggested that early in gestation, embryonic kidney possesses all the necessary precursors that compose the kidney vasculature well before arteriolar vessels can be discerned and proposed that those as yet unidentified precursors differentiate into all the cell types necessary for the development of the kidney arterioles, including endothelial cells, smooth muscle cells, and renin cells.[12,13] In this study however, we have shown morphologically that arteriolar vessels were seen in the early phase of glomerular formation before metanephric blast cells maturation.

Researchers have stated that it is now accepted that hematopoietic stem cells and endothelial cells originate during embryogenesis from a common progenitor, the hemangioblast.[14,15] These cells consist of a subpopulation of primitive streak mesoderm that migrates to the yolk sac where they establish the primitive hematopoietic system. These findings are in agreement with the morphological finding in our study.

Abrahamson has shown that their findings do

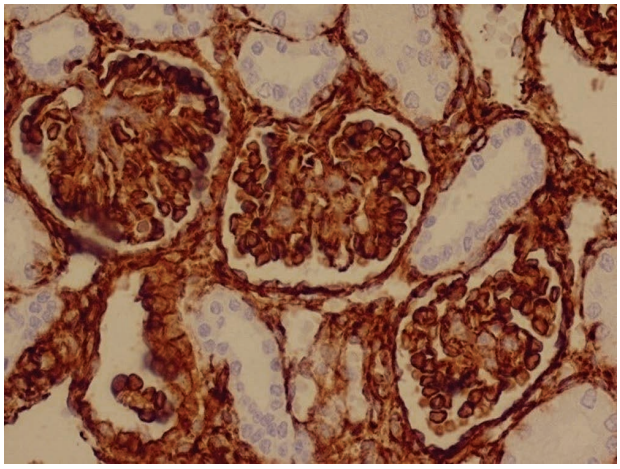

Figure 13. Vimentin diffusely stained glomerular, tubular components, and stroma (vimentin, × 400).

not rule out the possibility that kidney angioblasts might migrate into metanephrons from some external source.[4] On the other hand, angioblasts might also drive directly from the metanephric mesenchyme, as do all of the other epithelial cells of the nephron. There must also be some coordinated mechanism for connecting the developing renal microvasculature with the larger systemic blood supply. In our study, we showed this connection with presence of erythrocytes in the immature glomeruli and with continuity with the basement membrane of intima of afferent arteriole.

Nishimura and colleagues developed a method to detect the initial angiogenesis of dorsal aorta into metanephrons and clarified that dorsal aorta angiogenesis occurred at an early stage of metanephric development.[11] They also elucidated the role of dorsal aorta angiogenesis in promoting the early blood vessel formation and glomeruli maturation. It is suggested that blood flow and dynamic circulation of various factors at the early developing stage may be prerequisite to a successful construction of blood vessels in a complex organs either in vitro or in vivo. Overall, these findings contribute to a better understanding of dorsal aorta angiogenesis during kidney development and shed light on its significant value as it could be useful for tissue engineering of this complex organ. Based on this study, connection between dorsal aorta and metanephric blast cells are necessary for vascular development. Metanephric blastemal might therefore play a regulatory role in the angiogenesis as we hypothesized in this study.

CONCLUSIONS

This study shows that there is a relationship between the metanephric blast cells and major vessel critical for angiogenesis. When afferent arteriole come in contact with the immature glomeruli, its intima migrates into the glomerular tuft to form intraglomerular capillary system, while its smooth muscle remains at the entrance orifice and develops juxtaglomerular apparatus cells.

ACKNOWLEDGMENTS

We thank Dr Asghar Rastegar, Professor of Nephrology, Yale Medical School, for his suggestions and editing of this manuscript, and Mrs Z Shaykhshoaei for her great technical assistance.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Sanchez NC, Tenofsky PL, Dort JM, Shen LY. What is normal intra-abdominal pressure?/Discussion. American

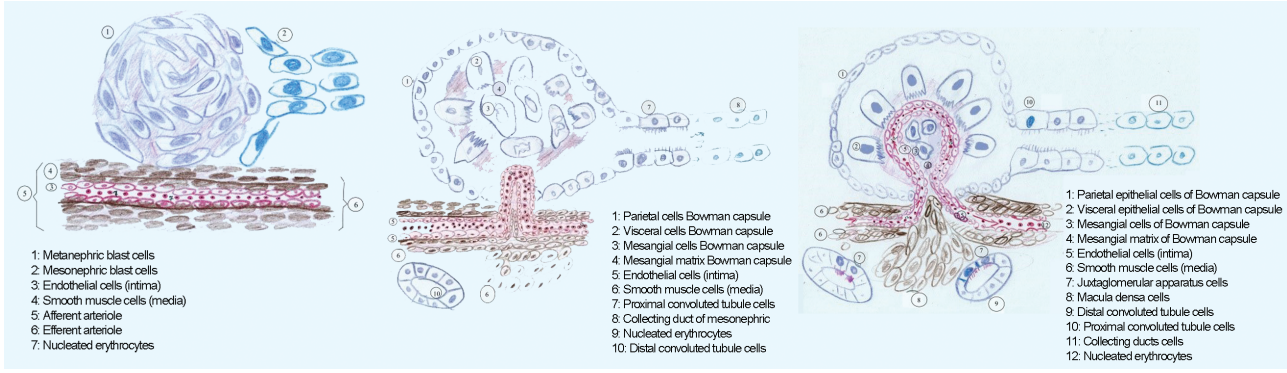

Figure 14. Top, The relationship between arteriole and metanephric blast cells; B, the rupture of parietal lining of the Bowman capsule allowing penetration of arteriolar intima into glomerular tuft; C, the protrusion of arteriolar intima forming glomerular capillary tuft. The smooth muscle of the arteriole then formed the juxtaglomerular apparatus.

Surg. 2001;67:243.

2. Sequeira Lopez ML, Gomez RA. Development of the renal arterioles. J Am Soc Nephrol. 2011;22:2156-65.
3. Sequeira-Lopez MLS, Lin EE, Li M, Hu Y, Sigmund CD, Gomez RA. The earliest metanephric arteriolar progenitors and their role in kidney vascular development. Am J Physiol Regul Integ Comp Physiol. 2015;308:R138-R49.
4. Abrahamson DR. Development of kidney glomerular endothelial cells and their role in basement membrane assembly. Organogenesis. 2009;5:1-13.
5. Kang D-H, Kanellis J, Hugo C, et al. Role of the microvascular endothelium in progressive renal disease. J American Soc Nephrol. 2002;13:806-16.
6. Sutton TA, Fisher CJ, Molitoris BA. Microvascular endothelial injury and dysfunction during ischemic acute renal failure. Kidney Int. 2002;62:1539-49.
7. Meier-Kriesche HU, Schold JD, Srinivas TR, Kaplan B. Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. Am J Transplant. 2004;4:378-83.
8. Lizardo-Daudt HM, Edelweiss MIA, Santos FTd, Schumacher RdCA. Diagnosis of the human fetal age based on the development of the normal kidney. J Brasil Patol Med Lab. 2002;38:135-9.
9. Vaughan MR, Quaggin SE. How do mesangial and endothelial cells form the glomerular tuft? J Am Soc Nephrol. 2008;19:24-33.
10. Faa G, Gerosa C, Fanni D, et al. Morphogenesis and molecular mechanisms involved in human kidney development. J Cell Physiol. 2012;227:1257-68.
11. Nishimura Y, Hsu HH, Wang PC. Detection of initial angiogenesis from dorsal aorta into metanephroi and elucidation of its role in kidney development. Regenerat Ther. 2016;4:27-35.
12. Hyink DP, Tucker DC, St John P, et al. Endogenous origin of glomerular endothelial and mesangial cells in grafts of embryonic kidneys. Am J Physiol Ren Physiol. 1996;270:F886-99.
13. Lopez MLSS, Pentz ES, Robert B, Abrahamson DR, Gomez RA. Embryonic origin and lineage of juxtaglomerular cells. Am J Physiol Ren Physiol. 2001;281:F345-56.
14. Lancrin C, Sroczynska P, Serrano AG, et al. Blood cell generation from the hemangioblast. J Mol Med. 2010;88:167-72.
15. Sabin FR. Origin and development of the primitive vessels of the chick and of the pig. Washington, DC: Carnegie Institution of Washington; 1917.

Correspondence to:
Mohammad Mehdi Moeini-Aghtaei, MD
Pathology and Stem Cell Research Center, Department of Pathology, Afzalipour Kerman University of Medical Sciences, Bahman 22th Blvd, PO Box: 444, Kerman, Iran
Tel: +98 34 3325 7112
E-mail: moini_m@yahoo.com

Received October 2016
Revised January 2017
Accepted January 2017