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Review

Prolonging Nephrogenesis in Preterm Infants
A New Approach for Prevention of Kidney Disease in Adulthood?

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Chronic kidney disease represents a dramatic worldwide resource-consuming problem. This problem is of increasing importance even in preterm infants, since nephrogenesis may go on only for a few weeks (4 to 6 weeks) after birth. Recent literature focusing on traditional regenerative medicine does not take into account the presence of a high number of active endogenous stem cells in the preterm kidney, which represents a unique opportunity for starting regenerative medicine in the perinatal period. Pluripotent cells of the blue strip have the capacity to generate new nephrons, improving kidney function in neonates and potentially protecting them from developing chronic kidney disease and end-stage renal disease in adulthood. There is a marked interindividual neonatal variability of nephron numbers. Moreover, the renal stem/progenitor cells appear as densely-packed small cells with scant cytoplasm, giving rise to a blue-appearing strip in hematoxylin-eosin–stained kidney sections ("the blue strip"). There are questions concerning renal regenerative medicine: among preliminary data, the simultaneous expression of Wilms tumor 1 and thymosin β4 in stem/progenitor cells of the neonatal kidney may bring new prospects for renal regeneration applied to renal stem cells that reside in the kidney itself. A potential approach could be to prolong the 6 weeks of postnatal renal growth of nephrons or to accelerate the growth of nephrons during the 6 weeks or both. Considering what we know today about perinatal programming, this could be an important step for the future to reduce the incidence and global health impact of chronic kidney disease.

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INTRODUCTION

Chronic kidney disease (CKD) represents a global public health issue with different features to take into account in different parts of the world.[1] The prevalence of end-stage renal disease (ESRD) is increasing dramatically during the last years.[2] According to the 2010 Global Burden of Disease study, CKD was ranked 27th in the list of causes of total number of global deaths in 1990, but rose up to 18th in 2010, with a annual death rate of 16.3 per 100 000.[3] Despite the magnitude of resources designated to the therapy of patients affected by ESRD and the improvements in the quality of dialysis therapy, these patients experience a reduced quality of life, associated with high mortality.[4] Children undergoing kidney transplantation, thanks to the improvements in antirejection therapy, have a 95% chance of surviving at 5 years, but their long-term survival rate lowers to 79% at 10 years and 66% at 20 years.[5] These data clearly indicate that kidney transplantation does not represent the definitive therapy for ESRD.

Regenerative medicine has made remarkable progress in recent years, with the development of techniques to mobilize and activate renal stem cells in injured kidneys or to introduce exogenous stem cells for tissue repair.[6] Unfortunately, when the complex kidney structure is disrupted by ESRD, traditional stem cell-based approach has been demonstrated to be unable to regenerate the damaged organ. Whole kidney "de novo" regeneration may be considered a promising therapeutic approach for patients with ESRD.[7] Taken together, these data suggest that change—discarding old approaches and giving opportunity to new therapies—is a mandate, focusing on prevention of CKD insurgence when the kidney architecture is still preserved. The theme of this article is the introduction of a new potential approach for the prevention of CKD, based on the utilization of a previously unrecognized physiological regenerative source: the huge amount of stem cells that are present in the kidney of preterm infants at birth.

VARIABILITY OF NEPHRON NUMBERS

The number of nephrons in the kidney of adults is characterized by a marked interindividual variability, ranging almost 9 fold from about 200 000 up to 1 800 000 or more, nephron burden being inversely related to body weight at birth.[8] Nephron number at birth is conditioned by the intrauterine environment, the 9 months of gestation permanently influencing the number of developed functional glomeruli. Since nephrogenesis ends around the 36th week of gestation, the nephron number detectable at birth will be stable during life. As a consequence, events occurring during the early steps of kidney development set lifelong trajectories, raising or lowering risk of developing kidney failure later in life. Considering 2 individuals at the extremes of the spectrum, carrying 200 000 and 1 800 000 nephrons respectively, the clinical significance of a noxa destroying 100 000 nephrons could be completely different in these individuals.[9] Preterm infants, given their low nephron number,[10] are considered at a higher risk of congenital oligonephronia, as well as low birth weight neonates, linking body weight at birth with the susceptibility to develop CKD in adulthood.[11]

PERINATAL KIDNEY

Kidneys in preterm newborns show relevant functional and structural differences as compared to term newborns. Kidney function in the neonate differs quantitatively from that in older children. Although a full complement of nephrons is present at 34 weeks of gestation, glomerular and tubular function continue to mature during the 1st year of life. In the human, nephrogenesis is complete at 36 weeks of fetal gestation, so that fullterm newborn infants have as many nephrons as they will have for the rest of their life, but a prematurely born infant will continue to produce new nephrons for sometime after birth (4 to 6 weeks), depending on the neonate gestational age. A marked increase in renal perfusion may contribute to these changes. Following delivery, the loss of placental renal blood flow is only 3% to 5% of the cardiac output, as compared to approximately 20% in the infants and children. The next phase of growth is the anatomical maturation of the nephrons already present. Thus, there are changes in glomerular and tubular histology that could theoretically affect kidney function. Included in this phase of renal growth are biochemical changes in the renal cells.[9-13] Despite its incomplete maturation, the newborn kidney is functional and glomerulotubular balance is intact. Thus, the presence of "immature" kidneys should not be used to explain disturbances in fluid and electrolyte balance or kidney dysfunction.

The presence of a huge amount of stem/progenitor cells in close proximity of the renal capsule represents the most important peculiar feature of the fetal kidney, and it is the focus of our work.[12] The renal stem/progenitor cells appear as densely-packed small cells with scant cytoplasm, giving rise to a blue-appearing strip in hematoxylin-eosin–stained kidney sections. After birth, nephrogenesis may go on only for 4 to 6 weeks, and then it stops.[12] As a consequence, in a preterm infant born at 26 weeks, nephrogenesis will stop for example at the 32nd postconceptional week, linking prematurity with oligonephronia and with higher susceptibility of preterm infants to develop kidney disease later in life. If this newborn has suffered intrauterine growth restriction in the womb or acute kidney injury postnatally, this interval of nephrogenesis will be even more reduced.

REGENERATIVE MEDICINE

Concept

The presence of a high number of active

endogenous stem cells in the preterm kidney represents a unique opportunity for starting regenerative medicine in the perinatal period. Pluripotent cells of the blue strip have the capacity to generate new nephrons, improving kidney functions in neonates and protecting them from developing CKD and ESRD in adulthood. The main obstacle for the actual utilization of this regenerative potential is the abrupt interruption of their nephrogenic activity a few weeks after birth in preterm infants, irrespective of the gestational age at birth. To understand how to switch on stem/progenitors of the blue strip, maintaining their activity in the generation of new nephrons till the 36th postconceptional week represents the new challenge for the prevention of CKD in adulthood. Dealing with endogenous stem cells physiologically present in the fetal kidney, we defined this new approach as "physiological renal regenerative medicine.[13,14]"

Open Questions Concerning Renal Regenerative Medicine

Some questions should be raised regarding this new approach to kidney regeneration early in life:

(1) Why researchers involved in kidney regeneration did not consider this relevant endogenous renal stem burden?
(2) Why the literature regarding kidney development has been exclusively focused on factors blocking nephrogenesis (brake) and not on factors favoring an optimal glomerulogenesis (accelerator)?
(3) Which is the way to follow for activating renal progenitors of the blue strip, forcing them to continue or accelerating nephron production till the 35th or 36th postconceptional week, reducing the gap in nephron number between preterm and at term newborns?

It is evident that traditional renal regenerative medicine is unable to solve the problem of kidney failure or to alleviate patients with ESRD. In our opinion, one of the major obstacles for a successful renal regeneration in adults has been the application of regenerative techniques to severely injured kidneys; moreover, the introduction of exogenous stem cells is unable to completely regenerate the totally disrupted renal tissue.[7] The major advantages of the application of regenerative techniques to newborn with a low nephron burden at birth are here listed:

(1) To avoid the ethical problems associated with the manipulation of germ cells in producing embryonic stem cells[15];
(2) To bypass the use of retroviral transduction in the generation of induced pluripotent stem cells and the risk related to our limited understanding of its effects[16];
(3) To avoid the use of donor bone marrow-derived stem cells, with limitations due to rarity of their migration into the affected kidney and to their limited ability to trans-differentiate[17];
(4) To avoid the use of mesenchymal stem cells from adipose tissue of CKD patients, often inappropriate for renal regeneration due to functional incompetence induced by uremia[18]; and
(5) To avoid the ethical issues related to the use of heterologous animals including porcine precursors in the efforts to regenerate "de novo" a whole functional kidney.[19]

The answer to our 2nd question regards the inability of perinatologists to evaluate all the factors that may influence the delicate equilibrium of kidney growth and development during gestation. Great attention has been devoted to the factors lowering the nephron number, which are able to modify the equilibrium between mitosis (accelerator) and apoptosis (brake), downregulating renal stem cell survival and differentiation, and ending with a modified kidney development.[20] In the opinion of the authors, more attention should be devoted to defining all factors stimulating stem cell survival and differentiation during gestation, which enable us to accelerate nephrogenesis particularly in the perinatal period. In this new approach, the developing kidney should be seen as the optimal source of stem/pluripotent cells to be implemented by favorable epigenetic factors, including adequate caloric intake and proper vitamins and normoxia, and avoiding nephrotoxic drugs, such as aminoglycosides, glycopeptides, nonsteriod anti-inflammatory drugs, and amphotericin.[21] The knowledge of all factors able to improve nephrogenesis after birth could represent a powerful tool in the hands of neonatologists, allowing them to restore nephron deficit in preterm infants and newborns with intrauterine growth retardation, the two clinical settings typically associated with congenital oligonephronia.

The 3rd question regards the possible clinical

approach aimed at ameliorating and prolonging nephrogenesis after birth. Our goal is the definition of a new target therapy, aimed at prolonging nephrogenesis after birth in all neonates with a low nephron burden. There is a crucial period postnatally where nephrogenesis continues in preterm neonates. This period is normally about 6 weeks long, but it can be shorter in newborns affected by acute kidney injury or with intrauterine growth retardation.

New Perspectives

Our proposal is based on a better comprehension of the molecular pathways regulating renal stem/progenitor cells survival and function in the perinatal period, with particular attention to preterm newborns.[22]

Recent evidence that paracrine stimulation underlies the functional benefits in cell transplantation led to a paradigm shift in regenerative medicine: from cell therapy to factor/protein-based regenerative therapy, future regenerative approaches likely involving the definition of synergistic protein cocktails specific for each organ.[23] The basis for the development of a new nephrogenic protein-based therapy is the definition of the protein markers expressed by the renal progenitors operating in the blue strip. Previous papers from our group showed that renal precursors show a peculiar immunohistochemical pattern as compared to stem/progenitor cells in other organs.[23] Immunohistochemistry of the nephrogenic zone is characterized by expression of Wilms tumor 1 (WT1),[24] paired box protein-2, CD44, B-cell lymphoma-2, thymosin β10,[25,26] thymosin β4 (Tβ4), and nucleotide sugar epimerase (personal unpublished data). Comprehensive profiling of human renal stem cells includes the expression of markers such as CD24, CD133, NCAM, and EpCAM.[27]

Among these preliminary data on the profiling of human renal stem cells, the contemporary expression of WT1 and Tβ4 in the stem/progenitor cells of the human embryo kidney deserves, in our opinion, some consideration regarding the research on stimuli that could initiate the formation of new nephrons in the neonatal kidney after birth. Thymosin β4 is expressed in multiple embryonic human tissues, including salivary glands,[28] the embryonic heart,[29] and the developing kidney.[30] In the embryo heart, Tβ4 is essential to initiate embryonic coronary developmental program by stimulating cardiomyocytes migration and promoting cardiac cell survival.[31] At molecular level, Tβ4 initiates protein kinase C inducing an organ-wide epicardial thickening and activation of progenitor cells, while initiating the expression of numerous pro-angiogenic genes.[32] The stem or progenitor cell population, thought to be derived from the epicardium, are targeted and activated by Tβ4 even in the adult heart, restoring their vascular potential via re-expression of the key embryonic epicardial gene, WT1, resulting in their mobilization and differentiation to give rise to de novo cardiomyocytes.[33] The beneficial effects of Tβ4 on cardiomyocytes regeneration is related to its ability to form a complex with integrin-linked kinase, resulting in the activation of survival kinase Akt, which stimulates production of vascular endothelial growth factor.[34] Cardiomyocytes derived from Tβ4-activated epicardial cells have been shown to functionally integrate with resident cardiomyocytes to regenerate functional myocardium.[35] Taken together, these data indicate that the simultaneous expression of WT1 and Tβ4 in stem/progenitor cells of the neonatal kidney may bring new prospects for renal regeneration applied to renal stem cells that reside in the kidney itself.

CONCLUSIONS

Chronic kidney disease represents a dramatic worldwide resource-consuming problem. It is difficult to define histologically what is normal and what is not, taking into the account the enormous variability of the number of nephrons at birth in apparently healthy individuals. All efforts must be promoted to enhance protective factors or minimize aggressive factors in preterm developing kidneys. A potential approach could be to prolong the 6 weeks of postnatal renal growth of nephrons or to accelerate the growth of nephrons during the 6 weeks or both. A tailored approach could be hypothesized in the future, taking into account all information on the kidney and renal regenerative medicine. In this setting, the simultaneous expression of WT1 and Tβ4 in stem/progenitor cells of the neonatal kidney may bring new prospects for renal regeneration applied to renal stem cells that reside in the kidney itself. Modulating these main actors in the renal

scenario, it is perhaps possible to look at old data with new approaches, favoring endogenous renal stem cell's mitosis, blocking apoptosis and giving the possibility to preterm newborns to improve their final number of nephron. Considering what we know today about perinatal programming, this could be an important step for the future to reduce incidence and global health impact of CKD.

CONFLICT OF INTEREST

None declared.

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