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"Physiological" renal regenerating medicine in VLBW preterm infants: could a dream come true?

Daniela Fanni[1], Clara Gerosa[1], Sonia Nemolato[1], Cristina Mocci[1], Giuseppina Pichiri[1], Pierpaolo Coni[1], Terenzio Congiu[2], Marco Piludu[3], Monica Piras[3], Matteo Fraschini[4], Marco Zaffanello[5], Nicoletta Iacovidou[6], Peter Van Eyken[7], Guido Monga[8], Gavino Faa[1] & Vassilios Fanos[9]

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An emerging hypothesis from the recent literature explain how specific adverse factors related with growth retardation as well as of low birth weight (LBW) might influence renal development during fetal life and then the insurgence of hypertension and renal disease in adulthood. In this article, after introducing a brief overview of human nephrogenesis, the most important factors influencing nephron number at birth will be reviewed, focusing on the "in utero" experiences that lead to an increased risk of developing hypertension and/or kidney disease in adult. Since nephrogenesis in preterm human newborns does not stop at birth, but it continues for 4–6 weeks postnatally, a better knowledge of the mechanisms able to accelerate nephrogenesis in the perinatal period, could represent a powerful tool in the hands of neonatologists. We suggest to define this approach to a possible therapy of a deficient nephrogenesis at birth "physiological renal regenerating medicine". Our goal in preterm infants, especially VLBW, could be to prolong the nephrogenesis not only for 6 weeks after birth but until 36 weeks of post conceptual age, allowing newborn kidneys to restore their nephron endowment, escaping susceptibility to hypertension and to renal disease later in life.

Keywords: Hypertension, kidney, nephrogenesis, renal disease

Introduction

A fascinating hypothesis has recently emerged from the literature suggesting that specific adverse exposures during fetal life, which cause growth retardation as well as low birth weight (LBW), may also influence renal development, and even play a role in the development of renal disease in adulthood [1]. Since an estimated 16% of births worldwide are LBW, including small for gestational age (SGA) neonates, the subsequent development of these children is critically important, for the future of the children themselves and also for the future of the countries they will inherit [2].

A significantly reduced number of nephrons at birth in preterms and in SGA newborns may be a risk factor for development of kidney disease in adulthood and, in particular, for development of hypertension later in life [3].

Since conflicting results have been published in recent years concerning the hypothesis of the fetal origin of adult kidney disease [4], this study was aimed at reviewing the most important data on the possible correlation between reduced nephron number, LBW and kidney disease in adult life [5].

Brief overview of nephrogenesis in humans

Three excretory organs appear during human development, the pronephros, the mesonephros and the metanephros (Figure 1). The pronephros develops at 21–22 days of gestation, from the coelom with which it maintains a direct communication. The pronephros may be considered one single large rudimentary glomerulus, composed by a cavity, the nephrocoel, encircling a unique filtration unit, the glomus, which projects into the nephrocoel. From the wall of nephrocoel a solid bud emerges and undergoes cavitation giving rise to multiple ducts which join the nephrocoel to the pronephric duct, the future Wolffian duct, and to the cloaca [6]. The pronephros undergoes regression around the 25th gestational day in humans, simultaneously with the appearance of the second excretory organ in human development, the mesonephros [7].

The mesonephros originates from the mesonephric mesenchyme which, under induction of the Wolffian duct, undergoes the process of mesenchymal-epithelial transition, giving rise to renal vesicles, the first mesonephric epithelial structure, which differentiates into a mature nephron. The mesonephros consists of multiple mature nephrons, about 40 in humans, composed of glomeruli with proximal and distal tubules, which fuse with the Wolffian duct, in the absence of loops of Henle [7]. Complete nephrons of the mesonephros produce small amounts of urine. By 33 days of gestation, the mesonephros reaches its maximum degree of development, and at the same time, starts its regression which in females is complete, whereas in males mesonephric tubules give rise to the efferent tubules of the

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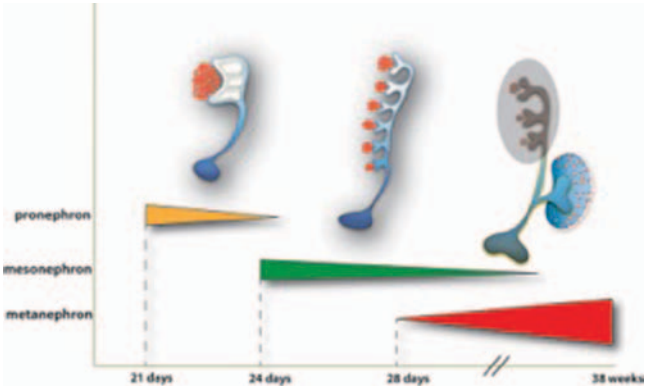

Figure 1. Excretory organs during human development: the pronephros, the mesonephros and the metanephros.

testis. Mesonephric remnants continue to exist till the last weeks of gestation, overlapping the developing metanephros.

Around day 30, the caudal region of the duct of Wolff gives rise to the ureteric bud, which proliferates and emerges from the Wolffian duct, invading the surrounding metanephric mesenchyme and giving rise to the third excretory organ in humans: the metanephros [8]. The branching ureteric bud invade the undifferentiated metanephric mesenchyme, which undergoes the same process of mesenchymal-epithelial transition previously experienced in the mesonephros. Undifferentiated metanephric mesenchymal cells condensate in the cap mesenchyme, which develops into the renal vesicle, the first mesenchyme-derived epithelial structure. The renal vesicle gives rise to the comma body, which gives rise to the S-shaped body, which originates the glomerulus, proximal and distal tubules, and Henle loops. The distal tubule eventually fuses with the collecting ducts, the only epithelial structure of the mature kidney which originates from the ureteric tree. The region of the ureteric bud external to the metanephric mesenchyme gives rise to the ureter [8].

Why do three excretory organs develop during human fetal life? And what is the association between them? Given that embryology does not give answers to any finalistic question, we may try to give an answer regarding the similarities and the differences between these sequential structures. The pronephros looks like a first tentative of the human embryo to develop an excretory unit. It is composed of one large glomerulus and by poorly differentiated tubuli which fuse with the mesonephric duct, a structure that, under different forms, will be the anchor-structure for all three excretory organs. The mesonephros appears to be a more complex organ, in which multiple nephrons are assembled together, organized in a orderly way, and producing urine which is excreted into the Wolffian duct. In the mesonephros, the building techniques, represented by the process of mesenchymal to epithelial transition, is first tried out. The same building technique will be subsequently utilized by the metanephros in the construction of the mature human kidney. The original oval bricks, represented by the metanephric mesenchymal cells, under the coordination of the ureteric bud tips, are transformed into square bricks, the epithelial progenitors of glomerular and tubular cells, which, joining together, give rise to an epithelial structure, the renal vesicle, that will differentiate into mature nephrons. The same building technique will be subsequently utilized by the metanephros in the construction of the mature human kidney. It thus appears that pronephros, mesonephros and metanephros are three sequential projects, in which a initial unique simulation of one excretory unit followed by a second simulation involving the definition of the building techniques and the construction of a series of more differentiated filtration units, followed by the metanephros as the final project, whose success depends on the building techniques tried out in the previous two projects.

Factors influencing nephron number at birth

There is a wide range in nephron number in human beings with apparently normal kidneys, ranging at least fourfold, from 331,000 up to 1,424,000, with a mean value of 617,000 [9]. More recent studies in human adult kidneys showed the total glomerular number ranging almost ninefold, from 210,000 up to 1,825,000, with a mean value of 784,909 [10,11]. In recent years, a strong link is emerging between intrauterine development and kidney disease occurring in adulthood, thus reinforcing the theory of a developmental origin of adult health and disease, including nephropaties [12]. The type and quantity of nutrition received by the fetus in the womb, drugs and infections the embryo and the fetus were exposed to, mother's health or stress during pregnancy, maternal hypertension, maternal undernutrition, placental insufficiency and other pathological conditions causing fetal hypoxia to the fetus, are all factors that can influence renal development and may predispose to renal disease in later life [13]. The assumption of the relationship between kidney development and susceptibility to undergo renal failure in adulthood is at the basis of the hypothesis that we could trace adult kidney behavior to the 9 months before birth [14]. According with the fetal programming hypothesis, the 9 months of gestation probably constitute the most important period of our life, permanently influencing the number of developed nephrons at birth, number that will be stable during all our life, and that will represent our strength against all nephrotoxic injuries when present in high number, or our weakness when our kidney will be characterized by a low nephron number (Figure 2). Obviously, nephrons must be functioning. Considering three subjects carrying kidneys with 200,000 nephrons, with 900,000 and with 2,500,000 nephrons respectively, the clinical significance of a noxa destroying 100,000 nephrons could be completely different in these subjects [14]. Decades of animal experiments show that events that occur in the earliest steps of development, before and after birth, set lifelong health trajectories. These events alter health trajectories to raise (or lower) risk of our society's most common chronic diseases, including chronic renal failure.

But how can events occurring in utero lead to an increased risk of developing kidney disease later in life? Epigenetic is probably the right answer. Considering kidney development and its relation to adult renal disease, it has been hypothesized that an insult taking place in a specific time window during development may cause a permanent alteration in kidney architecture and function, affecting nephron number, glomerular volume, tubular cell function, vascular permeability, interstitial cell function and so on [15]. Among the factors which could interfere with neprogenesis during fetal life, drug treatment of the mother should be considered: mothers of newborns with acute renal failure have been shown to have received more drugs during pregnancy and delivery [16]. Among drugs which probably have major embryo-fetal toxic effects, nonsteroidal anti-inflammatory drugs (NSAIDs) can cross the placenta, reach the fetal circulation, and cause a spectrum of changes in the kidneys of the offspring [17].

In humans, nephrogenesis is complete by week 34–36 of gestation. As a consequence, at term babies normally do not show signs of active nephrogenesis at birth. In LBW newborns, and particularly in preterms, nephrogenesis has been shown to

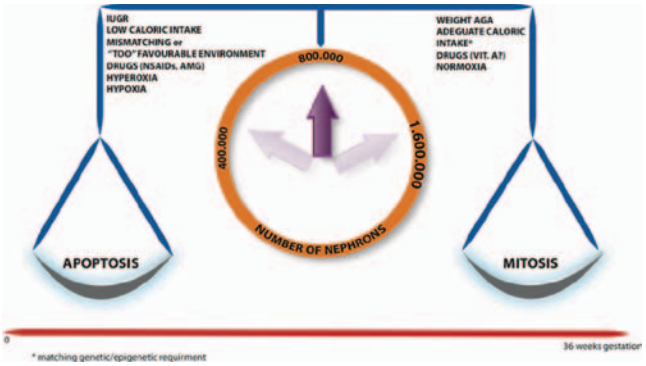

Figure 2. Factors that modify the delicate equilibrium between apoptosis and mitosis, determining nephron number at birth and postnatal nephrogenesis in preterm infants.

continue for some few time after birth, ending within about 6 weeks of postnatal life. This implies that our ability to restore low nephron number at birth by compensatory nephrogenesis after birth is very limited. As a consequence, all preterm infants, and LBW newborns, will have a low nephron number for the rest of their life, as compared to at term newborns. The recent finding, by our group, of a marked interindividual variability in nephron number among newborns with the same gestational age at birth, induce to consider other factors, other than gestational age, which might influence nephrogenesis and nephron number at birth [18].

It thus appears that a low nephron number at birth, so-called oligonephronia, is the principal factor linking epigenetic modulation of kidney development during gestation to the susceptibility to hypertension and chronic kidney injury in adult life. The exact mechanisms leading to oligonephronia and, eventually, to renal insufficiency in childhood and adulthood are not completely clarified yet. The hypothesis of a fetal origin of postnatal kidney disease is turning pregnancy into a scientific frontier, making the womb a very promising target for prevention, and raising hopes of overcoming chronic kidney disease through prophylactic interventions during gestation and in the perinatal period.

Possible mechanisms linking impaired neonatal nephrogenesis, low birth weight and adult kidney disease

The idea that nutrition may act during a critical window early in development to permanently affect or "program" long term health, first emerged with the work of McCoy in 1933 [19]. The hypothesis that nephron endowment at birth could be inversely related to the risk for developing essential hypertension in later life, was advanced in 1988 [20]. The Brenner hypothesis was based on the finding that nephron number in the normal population ranges from 300,000 up to 1,000,000 or more, suggesting that a congenitally low nephron number could represent a risk factor for hypertension. The Brenner hypothesis that nephron number may be programmed during gestation induced many researchers to investigate the factors that might influence kidney development during intrauterine life. LBW infants found to have fewer nephrons, i.e. congenital oligonephronia, providing a fitting and overlooked explanation for the eventual development of hypertension [21]. Further studies demonstrated a strong correlation between LBW and glomerular number, confirming that a decreased nephron numer could represent a risk factor for the development of renal hypertension and other kidney diseases [22]. The role of the intrauterine nutritional status in nephrogenesis was subsequently analyzed, suggesting that prenatal undernutrition may programe kidney development, having a major impact upon nephron number at birth as well as upon renal structure in later life [23]. Experimental studies carried out in sheep showed that late gestational placental insufficiency could lead, other than to growth restriction, to persistent reduction in arterial pressure, from birth to adulthood, resulting in systemic hypotension [24]. In other experimental studies, it has been shown that maternal limitations in nutrient supply and exposure of pregnant animals to elevated levels of hormones or toxins can lead to permanent deficit in nephron number, resulting in elevated blood pressure [25]. Epidemiological studies on several human populations have linked socioeconomic status with a suboptimal intrauterine environment, resulting in a nutrient-restricted fetus and a LBW infant who, later in life, will show an increased susceptibility to several diseases, including hypertension [26]. A possible link between LBW and development of renal disease in adulthood has been identified in the different glomerular size in LBW newborns. In humans with LBW, glomeruli have been found to be larger, a compensatory consequence of a reduction in total filtration surface area related to the reduced nephron number. Since the increased glomerular size is consistent with hyperfiltration, this could represent the link between nephron number and kidney disease. In fact, hyperfiltration represents a glomerular functional stress, able to accelerate glomerular senescence and loss of renal function, lowering in LBW infants renal reserve to adapt to dietary excesses or to compensate for kidney injury [27].

The mechanisms linking LBW and hypertension have been, subsequently, shown to be multifactorial, involving alterations in the normal regulatory systems involved in the long-term control of arterial pressure, and resulting in the fetal programming of hypertension [28]. In recent years, other studies have been carried out on the role played by fetal programming on susceptibility to develop hypertension and kidney diseases in adulthood: these studies were mainly focused on the causes of the reduced nephron number in LBW newborns. Several hypotheses have been put forward, including changes in DNA methylation, increased apoptosis in the developing kidney, alterations in the renin-angiotensin system activity [29], and fetal glucocorticoid exposure [30]. In particular, maternal protein restriction has been shown to suppress the newborn renin-angiotensin system and program adult hypertension in rats [31]. Taken together, the epidemiological studies carried out in different human populations, clearly demonstrate that the causal pathways linking LBW to the increased susceptibility to be affected by renal diseases in adult life seem to be complex and may include combined environmental and genetic mechanisms, working in sequence in various periods of life [4]. Two autopsy studies have clearly shown a lower nephron number in hypertensive subjects, associated with an enlargement of glomeruli indicating a hyperfiltration: the association of these factors could represent the cause of both high blood pressure and nephrosclerosis [32]. A major role to the whole perinatal nutritional status, including the postnatal nutritional environment, in the programming of adult hypertension has been recently demonstrated by a study in rats, in which a nephron deficit, prenatally induced by placental insufficiency, has been restored by correcting growth restriction during lactation [33]. The ability of an optimal lactation environment to restore nephron deficit and prevent hypertension in rats may be easily explained by the timing of nephrogenesis in this animal species, in which nephron production does not stop at birth, but goes on during all the lactation period, allowing kidneys to restore nephron endowment to the normal range. Unfortunately,

this ability could not be transferred to humans, nephrogenesis in human kidneys being characterized by an arrest before birth, normally around the 36th week of gestation. Breast feeding is the most efficient way to provide optimal nutrition. There is ongoing need to better understand the contribution of human milk in promoting renal health and development in preterm infants after birth. The question "how we do nourish this baby" in a 26 weeker even for the neonatal kidney must take into account that every maternal milk, whenever possible, should be tested and that metabolic requirements of the newborn are similar to those of the fetus. The goal should be to avoid "not enough" or "too much" nutrients, but, in clinical practice, this is not so easy to define.

The hypothesis of the association between intrauterine growth restriction and hypertension later in life has been criticized by other authors, who claimed that only circumstantial evidence is available to support the low nephron number/hyperfiltration/glomerular injury/systemic hypertension hypothesis, in the absence of a method to estimate glomerular number in living human beings. This method would allow to study conclusively the association between nephron endowment and blood pressure in humans [34].

The increased risk for developing chronic kidney disease and hypertension in adulthood, following an adverse fetal environment, is probably multifactorial, and includes gender differences. A role for sex hormones has been recently strongly suggested, leading to a lower incidence of hypertension in females compared with males [35]. According to the sex difference hypothesis, sex hormones might modulate activity of blood pressure regulatory systems, protecting females from systemic hypertension: this study provides critical support for the inverse relationship between birth weight and blood pressure, and introduces sex differences among the multiple factors involved in the fetal programming of hypertension.

In the rat, the early postnatal period has been confirmed to represent a critical time period for continued nephron development and for predisposing to hypertension in adulthood: growth restriction after birth was shown to reduce nephron number and increase blood pressure [36]. Even in this study, sex differences were considered, restriction of early postnatal growth increasing blood pressure in male rats. The effects of gestational age at birth on renal development and kidney growth has been studied in 243 SGA infants. In full-term and near-term infants, the relative kidney length was similar to or even higher than that of a control group of appropriate for gestational age infants; on the contrary, in smaller preterm babies, with less than 36 weeks of gestational age at birth, the relative kidney length was impaired up to the second year of life [5]. This study introduces a new element in the multifactorial pathogenesis of fetal programming of adult hypertension: gestational age at birth. On the basis of these data, we may speculate that, in spite of a reduced body weight at birth, near-term SGA babies had enough time to produce a nephron number sufficient for kidney function, and able to protect them against hypertension. On the other hand, in small babies born before the 36th week of gestation, the negative effects on nephrogenesis due to growth restriction should be added to the premature stop in nephrogenesis after birth, resulting in a severe reduction of the nephron number and in an increased susceptibility to develop hypertension. The assumption that a LBW is one, but certainly not the only, predictor of nephron endowment has been confirmed by animal models in which the mother is exposed to elevated glucocorticoids: the offspring shows a 20–40% reduction in nephron endowment, in the absence of relevant changes in the body weight at birth [37]. These data suggest that nephron number at birth may be associated with, but also dissociated from birth weight, and lays stress on the complexity of the developmental programming of kidney disease and hypertension in adulthood, that surely recognizes multiple factors and might mot be restricted to birth weight. The association of genetic factors with several environmental conditions has been indicated as the very likely condition at the basis of a modified embryonic-fetal development, subsequently constituting a health burden in later life [38]. In the same study, the role of postnatal factors are underlined, including the use of angiotensin-converting enzyme inhibitors in children with reduced nephron number and with glomerular hypertrophic changes. Debate exists around the potential independent role of postnatal growth acceleration in newborns showing fetal growth retardation at birth, and renal, cardiovascular and metabolic diseases in later life [1]. Specific attention has been paid to the timing of acceleration in growth and its potential association with adverse outcomes in later life: in particular, some growth trajectories might be associated with insurgence of renal disease in adulthood [39]. On the basis of these studies, specific vulnerable periods during prenatal and postnatal development could be identified, revealing time frames for early interventions able to prevent the development of kidney disease and hypertension in later life. Maternal nutrition has been recognized, in recent studies, as one of the most important factors that could interfere with nephrogenesis in humans, resulting in nephron underdosing followed by maladaptive glomerular changes, i.e. glomerular hyperfiltration and glomerular enlargement. Since the most rapid phase of human nephrogenesis occurs between the 20th and the 36th week of gestation, maternal malnutrition in this period has been identified as a major risk factor for determining a low nephron number and for development of arterial hypertension in later life [40]. The role of postnatal nutrition has been confirmed by recent studies as a relevant risk factor with a significant influence on long-term renal health in humans [27]. Singhiel A has proposed, for cardiovascular disease, that postnatal growth acceleration could explain in part adverse programming effects in infants born SGA (who show "catch up" growth immediately after birth) and long-term benefits in babied breast-fed (who are relatively undernourished and have slower growth in comparison with those formula-fed [41]. According with these data, postnatal growth and nutrition are emerging are important and potentially modifiable factors, and a optimal therapeutic target for the prevention of higher blood pressure or reduced renal function later in life.

Future perspectives

Human nephrogenesis is highly complex, and involves many cell types often transforming one into the other. Unraveling its mechanisms necessitates collaboration between multiple specialists, including gynecologists, neonatologists, biologists, nephrologists, biochemists, embryologists, pathologists, informatic engineers, and transmission and scanning electron microscopists. Four projects are currently under way in our group: scanning electron microscopy of the developing human kidney; development of a new method for an easy and reproducible nephron count; comparison of the morphological sequence of nephrogenesis in different animal species; immunostaining of fetal kidney in different phases of development.

Scanning electron microscopy

The sequence of events leading to the differentiation of podocyte precursors into mature podocytes in the developing kidney has

not been completely defined yet. We applied the osmium maceration method previously described by Riva A [42] to the study of fetal and newborn kidneys. Our preliminary data at scanning electron microscopy after osmium maceration, show the imature glomerulus as a 50 micron in diameter globoid structure, completely covered by roundish nuclei of podocyte precursors. At higher power, on the surface of immature glomeruli, it was possible to study the morphological sequence of differentiation of podocyte precursors. The differentiation of podocytes is characterized by nucleus flattening and cytoplasmic extensions originated by the cell body, embracing the underlying capillary tuft. In a second step, multiple branching digitiform processes originate from the cytoplasmic extensions of podocytes, giving rise to foot processes (Figure 3).

Determination of the total nephron number

This appears to be so important in the programming of adult health and renal disease. Unfortunately, no simple method is available for a correct estimation of nephron number based on the observation of an histological kidney sample. Even if it is widely accepted that the total number of nephrons in a kidney represents an important index of renal structure and health, a universally accepted method to determine the number of nephrons is still lacking. Several studies tried to address the problem with different methodological approaches (stereological counting methods and dissector methods) but the main problem still remains the high interindividual variability in nephron counting performed on a single tissue section. We do expect that the application of modern image processing and machine learning tools for automatic detection, segmentation, shape and textural characterization of histologic images could be of great help to address this important and difficult issue. Furthermore, we are developing novel computer aided algorithms for obtaining the total kidney nephron number, on the basis of the count of glomeruli observed in one kidney section, in order to complement the opinion of the pathologist.

Understanding the sequential steps of nephrogenesis

These steps have been generally considered similar in all mammals. Recently, our group has shown that nephrogenesis in pigs is marked different from humans. In particular, a previously unrecognized developmental structure, the composite tubuloglomerular nodule, has been identified in the developing porcine kidney [43]. On the basis of this new finding, the morphological sequence of nephrogenesis is under study in our group, with the aim of detecting alternative ways to glomerulogenesis and tubulogenesis to the human way of renal development.

Immunostaining and fetal kidney

Studies on immunoreactivity for different markers putatively involved in the process of mesenchymal to epithelial transition leading to nephron development, started two years ago with the finding that thymosin beta 10, a small peptide involved in many cellular functions, is highly expressed in fetal kidney during nephrogenesis [44]. In that study, thymosin beta 10 was mainly detected in the comma- and S-shaped bodies, the two structures originating from the renal vesicle giving rise to glomeruli,

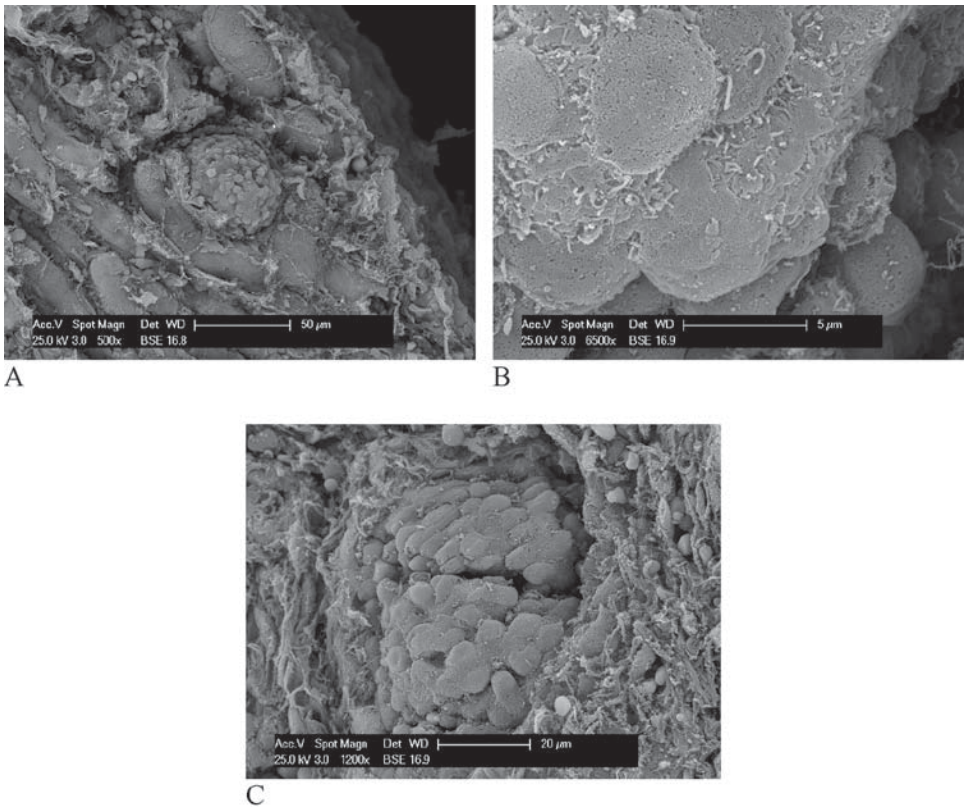

Figure 3. Scanning electron microscopy. (A) A panoramic view of the renal cortical, close to the capsule, showing distal and proximal convolute tubules and blood vessels. In the center there is a renal corpuscle with the glomerulus in open view with roundly shaped podocytes reminding a cluster of grapes It is not completely mature yet. The visceral layer is formed by podocytes and there is no evidence of well-formed blood vessels. (B) Detail of the podocyte layer. Few short processes start to expand from the cell body. Some pedicelli intermingle with the closer ones. The podocytes start to follow the structure of the forming blood vessels. (C) A higher view of the former renal corpuscle. Once removed the parietal layer, we can see the podocyte layer. Podocytes are closely packed and poligonally-shaped. There is also initial evidence of narrow hollow spaces in between.

proximal and distal tubuli. Further studies evidenced immunostaining for MUC1, a marker of epithelial differentiation, in a subset of nodular aggregates of cap mesenchymal cells, probably marking those nodules committed to undergo mesenchymal-epithelial transition [45]. In another study from our group, CD10 was found to be highly expressed in the undifferentiated metanephric mesenchyme, as well as in the glomerular epithelium and in proximal tubular cells [46]. The strong expression of CD10 in all stages of kidney development supports the hypothesis that CD10 could play a significant role in human nephrogenesis. Immunoreactivity for Wilms tumor 1 (WT1) was studied in fetal kidneys of different gestational age [47]. This zinc finger protein was detected in areas of active nephrogenesis and, in particular, WT1 appeared strongly expressed in developing and mature podocytes. These data indicate a role for WT1 in the initial phases of nephrogenesis, and in differentiation and maturation of podocytes. These data suggest a role for WT1 in the initial phases of nephrogenesis, and in differentiation and maturation of podocytes. Further studies are clearly needed to better characterize the cells of the developing kidney in order to gain more insight in the complex process of nephrogenesis in humans.

Transmission electron microscopy and immunoelectronmicroscopy

The ultrastructural features of developing progenitor cells have not been well characterized yet, both in humans and in experimental animals. The specific challenge of this research is to characterize by means at ultrastructural level, the morphological and immunohistochemical changes occurring during kidney development in metanephric mesenchymal cells during the different steps of the process of mesenchymal-epithelial transition. Moreover, the underlying molecular mechanisms and the intracellular changes occurring during the differentiation of glomerular and tubular cells will be characterized (Figure 4). To date, more electron microscopic work has been done on the kidney from adults than from newborns where the kidney differentiation events are not completely understood and remain to be ascertained. The proposed study employs high resolution transmission electron microscopy (TEM) combined with immunocytochemical methods in order to analyse the developmental expression of several translation products involved in the molecular mechanisms that regulate nephrogenesis. Samples of renal tissues from newborns of several animal species (mouse, rat) will be collected, placed in fresh fixative solution and processed by standard methods for embedding in resin. Thin sections will be cut from selected areas, collected on grids and examined in a TEM. For immunogold labeling thin sections will be immunolabeled using well-characterized antibodies to specific biomarkers and gold-labeled secondary antibodies and examined in a T EM. Quantitative analyses will be performed and statistical analyses of immunogold labeling densities will be done using appropriate post-hoc tests. Applying immunoelectronmicroscopy, we will also try to characterize the subcellular localization of the proteins involved in nephrogenesis, paralleling the previously reported immunohistochemical studies performed on paraffin sections. The originality of this research consists in determining the expression of specific signaling proteins during cytodifferentiation, looking for specific interactions among the signaling pathways and identifying protein constituents that can be monitored as potential "markers" during nephron differentiation process.

Renal cell culture

In vitro experiments will be utilized in order to study the effects of multiple substances on cell proliferation and differentiation. Even

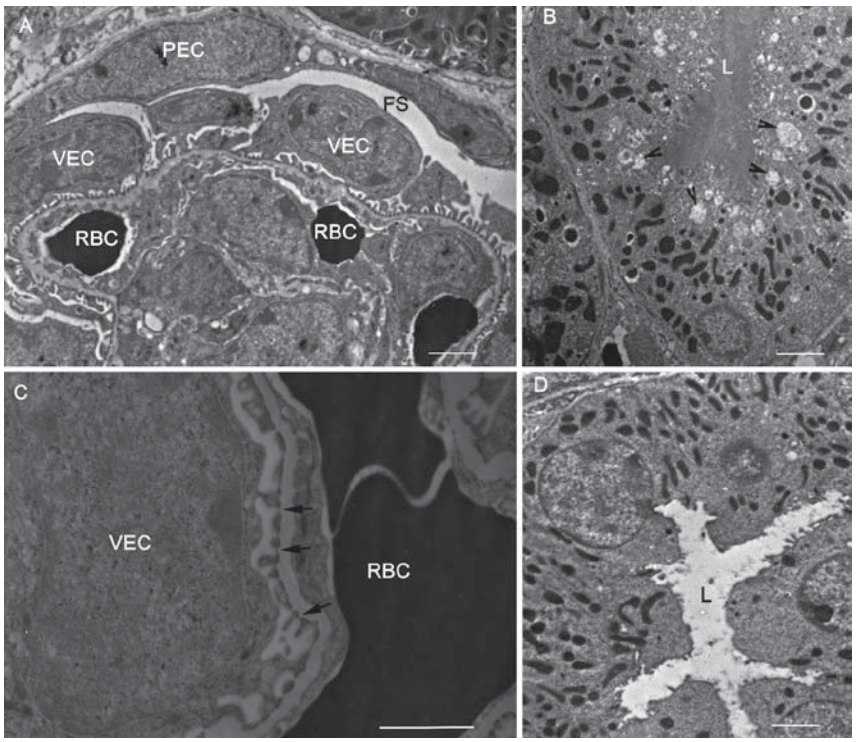

Figure 4. Ultrastructure of 4 days old rat kidney. (A) Electron microscopic overview of the glomerulus. Parietal epithelial cells (PEC), Visceral epithelial cells (VEC), Red blood cells (RBC). Filtration (Bowman's) space (FS). (B) Portions of renal tubules. The presence of evident vacuoles (arrowheads) in the apical portions of the cells may represent resorbed material due the abnormal permeability of the newborn glomeruli. Lumen (L). Bars= 2 μm. (C) Detail of glomerulus capillary wall. Note the presence of delicate connections between foot processes (arrows) of the visceral epithelial cells (VEC). Red blood cells (RBC). (D) Portions of renal tubules showing irregular apical microvilli and numerous mitochondria in the basilar compartments.

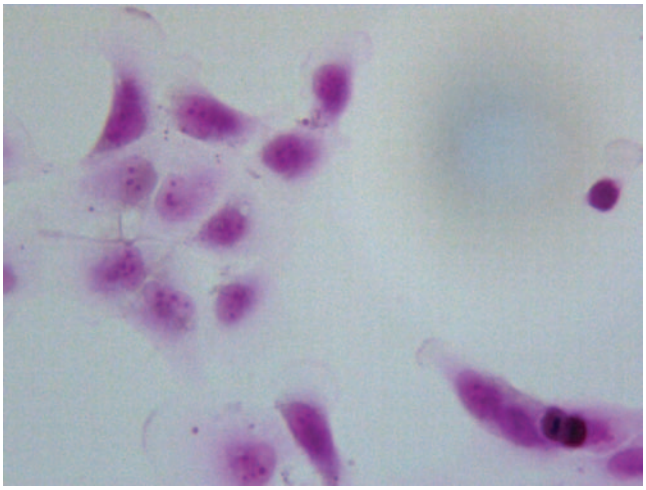

Figure 5. Cell line of human kidney carcinoma.

if cell lines do not necessarily correspond exactly to a distinct segment of nephron, they may retained some of the *in vivo* characteristics. For this reason, they have been used as a model in renal biology research. Different types of renal cells lines derived from human embryonal kidney, from human kidney carcinoma (Figure 5) and from tubular kidney will be used in order to study mechanisms involved in renal cell growth. In particular, substances able to modulate cell proliferation and apoptosis will be investigated in these kidney cell lines. Immunohistochemistry, electron immunohistochemistry and molecular biology results obtained *in vitro* will be paralleled with those obtained *in vivo* studies.

Molecular biology

Numerous factors expressed in a specific spatial and temporal pattern during Nephrogenesis have been recently studied at molecular level in humans as well as in animal models. In this study, the pattern of expression at RNA level of different gene involved in human nephrogenesis will be monitored in Real Time. In particular, messenger RNA of Timosin Betta 4, Timosin Betta 10, Muccin 1 and WT1 will be studied in human and in rat samples obtained from fetuses, infants or newborns as well as in cultured cells. RNA will be isolated using commercial Kits from frozen samples and real time will be performed using specific kits with syber green.

Conclusions

During the last years, numerous advances have been made in developmental nephrology. LBW has been associated with long-term alterations in renal function and blood pressure. These alterations can only be detected following these patients up to adulthood [48]. However, some concepts must be underlined. There is a crucial period, postnatally, where nephrogenesis seems to continue in preterm infants. This period is normally about 6 weeks, but it can be shorter in case of acute kidney injury. For example, a newborn born at 24 weeks of gestation could increase her/his nephron number only until 30 weeks of postconceptional age.

In the neonatologic and nephrologic literature, great attention has been devoted to the factors lowering the number of nephrons, altering the delicate equilibrium between apoptosis ("brake") and mitosis ("accelerator"), and limiting cell survival during renal development [49]. Some of these factors are well known, such as: hypo-malnutrition, mismatching nutrition with passage from an unfavorable environment to a "too favorable" environment, deficit or excess of oxygen supply, nephrotoxic drugs. However, in the opinion of the authors, more attention should be focused on defining, evaluating and promoting the factors stimulating mitosis and nephrogenesis and inhibiting apoptosis (Figure 2). From many years we know that apoptosis is regulated by agents both intrinsic, including the protoncogene b-cell lymphoma/leukemia gene product-2 (bcl-2), and extrinsic, including epidermal growth factor (EGF) [50]. A thorough understanding of the control of renal apoptosis during development and recovery of acute kidney injury (i.e. ischemic) may lead to new therapies to prevent or ameliorate abnormalities of kidney formation, to enhance regeneration following acute kidney injury, and to slow the progression of renal failure [51].

In fact experimental data clearly indicate that a better knowledge of the mechanisms able to accelerate nephrogenesis in the perinatal period, could represent a powerful tool in the hands of neonatologists: in rats, for example, an optimal lactation has been shown to correct growth restriction due to uteroplacental insufficiency, restoring nephron deficit and, eventually, preventing hypertension [33].

We suggest to define this approach to a possible therapy of a deficient nephrogenesis at birth "physiological renal regenerating medicine". Our interest in front of a newborn baby with a low nephron endowment cannot be limited to simply understand the factors which restricted fetal growth and impaired kidney development. That's epigenetics, things written in pencil you can change, differently from genetics, things written in pen you can't change. Our goal in preterm infants, especially VLBW could be to prolong the nephrogenesis not only for 6 weeks after birth but until 36 weeks of postconceptual age, allowing newborn kidneys to restore their nephron endowment, escaping susceptibility to hypertension and to renal disease later in life. Alternatively, an increase of nephron number in the first 6 weeks of postnatal life should be programmed. Further studies are needed to verify if this goal is utopia or a dream come true. In particular intervention trials are needed to evaluate the impact of changing a combination of pre- and post-natal factors on renal health of newborns and children.

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References

1. Geelhoed JJ, Jaddoe VW. Early influences on cardiovascular and renal development. Eur J Epidemiol 2010;25:677–692.
2. Grantham-McGregor SM. Small for gestational age, term babies, in the first six years of life. Eur J Clin Nutr 1998;52 Suppl 1:S59–S64.
3. Hotoura E, Argyropoulou M, Papadopoulou F, Giapros V, Drougia A, Nikolopoulos P, Andronikou S. Kidney development in the first year of life in small-for-gestational-age preterm infants. Pediatr Radiol 2005;35:991–994.
4. Jaddoe VW, Witteman JC. Hypotheses on the fetal origins of adult diseases: contributions of epidemiological studies. Eur J Epidemiol 2006;21:91–102.
5. Drougia A, Giapros V, Hotoura E, Papadopoulou F, Argyropoulou M, Andronikou S. The effects of gestational age and growth restriction on compensatory kidney growth. Nephrol Dial Transplant 2009;24:142–148.
6. Moritz KM, Wintour EM, Black MJ, Bertram JF, Caruana G. Factors influencing mammalian kidney development: implications for health in adult life. Adv Anat Embryol Cell Biol 2008;196:1–78.
7. Ludwig KS, Landmann L. Early development of the human mesonephros. Anat Embryol 2005;209:439–447.
8. Faa G, Gerosa C, Fanni D, Monga G, Zaffanello M, Van Eyken P, Fanos V. Morphogenesis and molecular mechanisms involved in human kidney development. J Cell Physiol 2012;227:1257–1268.

9. Nyengaard JR, Bendtsen TF. Glomerular number and size in relation to age, kidney weight, and body surface in normal man. Anat Rec 1992;232:194–201.
10. Hughson M, Farris AB 3rd, Douglas-Denton R, Hoy WE, Bertram JF. Glomerular number and size in autopsy kidneys: the relationship to birth weight. Kidney Int 2003;63:2113–2122.
11. Hoy WE, Douglas-Denton RN, Hughson MD, Cass A, Johnson K, Bertram JF. A stereological study of glomerular number and volume: preliminary findings in a multiracial study of kidneys at autopsy. Kidney Int Suppl 2003;83:S31–S37.
12. Barker DJ. Developmental origins of adult health and disease. J Epidemiol Community Health 2004;58:114–115.
13. Maringhini S, Corrado C, Maringhini G, Cusumano R, Azzolina V, Leone F. Early origin of adult renal disease. J Matern Fetal Neonatal Med 2010;23 Suppl 3:84–86.
14. Faa G, Gerosa C, Fanni D, Nemolato S, Monga G, Fanos V. Kidney embryogenesis: how to look at old things with new eyes. In: Fanos V, Chevalier RL, Faa G, Cataldi L, editors. Developmental Nephrology: from Embryology to Metabolomics. Quartu S. Elena: Hygeia Press; 2011. pp 23–45.
15. Puddu M, Cataldi L, Faa G, Yurdakok M, Maringhini S, Fanos V. Perinatal programming: long-term consequences for the kidney. In: Fanos V, Chevalier RL, Faa G, Cataldi L, editors. Developmental Nephrology: from Embryology to Metabolomics. Quartu S. Elena: Hygeia Press; 2011.
16. Cataldi L, Leone R, Moretti U, De Mitri B, Fanos V, Ruggeri L, Sabatino G, et al. Potential risk factors for the development of acute renal failure in preterm newborn infants: a case-control study. Arch Dis Child Fetal Neonatal Ed 2005;90:F514–F519.
17. Antonucci R, Zaffanello M, Pilloni MD, Fanos V. Antenatal non-steroidal anti-inflammatory drugs and the neonatal kidney. In: Fanos V, Chevalier RL, Faa G, Castaldi L, editors. Developmental Nephrology: from Embryology to Metabolomics. Quartu S. Elena: Hygeia Press;2011. pp 115–129.
18. Faa G, Gerosa C, Fanni D, Nemolato S, Locci A, Cabras T, Marinelli V, et al. Marked interindividual variability in renal maturation of preterm infants: lessons from autopsy. J Matern Fetal Neonatal Med 2010;23 Suppl 3:129–133.
19. McCoy GW. Activities and Trends in the Laboratory Field. Am J Public Health Nations Health 1933;23:318–322.
20. Brenner BM, Garcia DL, Anderson S. Glomeruli and blood pressure. Less of one, more the other? Am J Hypertens 1988;1:335–347.
21. Mackenzie HS, Lawler EV, Brenner BM. Congenital oligonephropathy: The fetal flaw in essential hypertension? Kidney Int Suppl 1996;55:S30–S34.
22. Mañalich R, Reyes L, Herrera M, Melendi C, Fundora I. Relationship between weight at birth and the number and size of renal glomeruli in humans: a histomorphometric study. Kidney Int 2000;58:770–773.
23. Langley-Evans SC, Langley-Evans AJ, Marchand MC. Nutritional programming of blood pressure and renal morphology. Arch Physiol Biochem 2003;111:8–16.
24. Louey S, Cock ML, Harding R. Postnatal development of arterial pressure: influence of the intrauterine environment. Arch Physiol Biochem 2003;111:53–60.
25. Kett MM, Bertram JF. Nephron endowment and blood pressure: what do we really know? Curr Hypertens Rep 2004;6:133–139.
26. Gilbert JS, Cox LA, Mitchell G, Nijland MJ. Nutrient-restricted fetus and the cardio-renal connection in hypertensive offspring. Expert Rev Cardiovasc Ther 2006;4:227–237.
27. Luyckx VA, Brenner BM. Low birth weight, nephron number, and kidney disease. Kidney Int Suppl 2005;97: S68–S77.
28. Alexander BT. Fetal programming of hypertension. Am J Physiol Regul Integr Comp Physiol 2006;290:R1–R10.
29. Zandi-Nejad K, Luyckx VA, Brenner BM. Adult hypertension and kidney disease: the role of fetal programming. Hypertension 2006;47:502–508.
30. Seckl JR, Meaney MJ. Glucocorticoid programming. Ann N Y Acad Sci 2004;1032:63–84.
31. Woods LL, Ingelfinger JR, Nyengaard JR, Rasch R. Maternal protein restriction suppresses the newborn renin-angiotensin system and programs adult hypertension in rats. Pediatr Res 2001;49:460–467.
32. Beaufils M. [Is hypertension related to the number of nephrons?]. Arch Mal Coeur Vaiss 2006;99:693–696.
33. Wlodek ME, Mibus A, Tan A, Siebel AL, Owens JA, Moritz KM. Normal lactational environment restores nephron endowment and prevents hypertension after placental restriction in the rat. J Am Soc Nephrol 2007;18:1688–1696.
34. Schreuder MF, Nauta J. Prenatal programming of nephron number and blood pressure. Kidney Int 2007;72:265–268.
35. Grigore D, Ojeda NB, Alexander BT. Sex differences in the fetal programming of hypertension. Gend Med 2008;5 Suppl A:S121–S132.
36. Wlodek ME, Westcott K, Siebel AL, Owens JA, Moritz KM. Growth restriction before or after birth reduces nephron number and increases blood pressure in male rats. Kidney Int 2008;74:187–195.
37. Moritz KM, Singh RR, Probyn ME, Denton KM. Developmental programming of a reduced nephron endowment: more than just a baby's birth weight. Am J Physiol Renal Physiol 2009;296:F1–F9.
38. Puddu M, Fanos V, Podda F, Zaffanello M. The kidney from prenatal to adult life: perinatal programming and reduction of number of nephrons during development. Am J Nephrol 2009;30:162–170.
39. de Beer M, van Eijsden M, Vrijkotte TG, Gemke RJ. Early growth patterns and cardiometabolic function at the age of 5 in a multiethnic birth cohort: the ABCD study. BMC Pediatr 2009;9:23.
40. Benz K, Amann K. Maternal nutrition, low nephron number and arterial hypertension in later life. Biochim Biophys Acta 2010;1802:1309–1317.
41. Singhal A. Does early growth affect long-term risk factors for cardiovascular disease? Nestle Nutr Workshop Ser Pediatr Program 2010;65:55–64; discussion 64.
42. Riva A, Congiu T, Faa G. The application of the OsO4 maceration method to the study of human bioptic material. A procedure avoiding freeze-fracture. Microsc Res Tech 1993;26:526–527.
43. Gerosa C, Fanos V, Fanni D, Nemolato S, Locci A, Xanthos T, Papalois A, et al. Toward nephrogenesis in the pig kidney: the composite tubulo-glomerular nodule. J Matern Fetal Neonatal Med 2011;24 Suppl 2:52–54.
44. Gerosa C, Fanni D, Nemolato S, Locci A, Marinelli V, Cabras T, Messana I, et al. Thymosin beta-10 expression in developing human kidney. J Matern Fetal Neonatal Med 2010;23 Suppl 3:125–128.
45. Fanni D, Fanos V, Monga G, Gerosa C, Nemolato S, Locci A, Van Eyken P, et al. MUC1 in mesenchymal-to-epithelial transition during human nephrogenesis: changing the fate of renal progenitor/stem cells? J Matern Fetal Neonatal Med 2011;24 Suppl 2:63–66.
46. Faa G, Gerosa C, Fanni D, Nemolato S, Marinelli V, Locci A, Senes G, et al. CD10 in the developing human kidney: immunoreactivity and possible role in renal embryogenesis. J Matern Fetal Neonatal Med 2012;25:904–911.
47. Fanni D, Fanos V, Monga G, Gerosa C, Locci A, Nemolato S, Van Eyken P, Faa G. Expression of WT1 during normal human kidney development. J Matern Fetal Neonatal Med 2011;24 Suppl 2:44–47.
48. Kwinta P, Klimek M, Drozdz D, Grudzien A, Jagla M, Zasada M, Pietrzyk JJ. Assessment of long-term renal complications in extremely low birth weight children. Pediatr Nephrol 2011;26:1095–1103.
49. Carev D, Krnic D, Saraga M, Sapunar D, Saraga-Babic M. Role of mitotic, pro-apoptotic and anti-apoptotic factors in human kidney development. Pediatr Nephrol 2006;21:627–636.
50. Coles HS, Burne JF, Raff MC. Large-scale normal cell death in the developing rat kidney and its reduction by epidermal growth factor. Development 1993;118:777–784.
51. Hammerman MR. Regulation of cell survival during renal development. Pediatr Nephrol 1998;12:596–602.