A new therapy for kidney injury: regeneration

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Abstract. – Because of progressive population ageing and epidemic diffusion of type 2 diabetes mellitus in industrialized Countries, we are attending a growing incidence of end stage renal disease. This phenomenon has induced researchers to study potential alternative methods of renal function replacement. Actually, only dialytic methodologies and renal transplant make possible survival of patients with terminal uremia, but both these therapeutic approaches show important limitations. The ideal solution would be represented by the possibility to “regenerate” the injured organ. This is the purpose of Regenerative Nephrology, a new medical domain which tries to develop new therapies through stimulation and induction in humans of regenerative processes already observed in other species, like reptiles and fishes. Such an ambitious and fascinating purpose requires a deep knowledge of the intricate networks which regulate the production of the hormones and mediators involved in the tissue regenerative processes. In this field the kidney embryonic development phases can represent a fundamental study model to acquire information about the reparative mechanisms of the structure and function of this excretory organ.

Key Words: Regenerative nephrology, Cytokines, Stem cells.

Abbreviations

GBM = glomerular basement membrane;
PDGF = Platelet-derived growth factor
SD = Slit diaphragm
VEGF-A = vascular endothelial growth factor A
VEGF-C = vascular endothelial growth factor C
VEGFR2 = vascular endothelial growth factor receptor 2
UB = ureteric bud
MM = metanephric mesenchyme
EGF = epidermal growth factor
TGF-α = transforming growth factor-α
TGF-β = transforming growth factor-β
EGFR = epidermal growth factor receptor
HB-EGF = heparin-binding epidermal growth factor
Wnt-4 = wingless-type MMTV integration site family, member 4
HGF = hepatocyte growth factor
MDCK = Madine-Darby canine kidney
ECM = extracellular matrix
BMP = morphogenetic proteins
LRTC s = label-retaining tubular cells
UUO = unilateral ureteral obstruction
BrdU = bromodeoxyuridine
CD24 = cluster of differentiation 24
CD133 = cluster of differentiation 133
BM = bone marrow
HSCs = hematopoietic stem cells
MSCs = mesenchymal stromal cells
CSF-1 = colony-stimulating factor 1
HA = hyaluronan
CD44 = cluster of differentiation 44
EMT = epithelial-mesenchymal transdifferentiation
AQP-1 = aquaporin 1
AQP-2 = aquaporin 2
Wt1 = Wilms tumor 1
Pax2 = paired box 2
Eya1 = eyes absent homolog 1
Six1 = SIX homeobox 1
Six2 = SIX homeobox 2
Osr1 = odd-skipped related 1
Sall1 = sal-like 1
GDNF = glial cell line-derived neurotrophic factor
FGF = fibroblast growth factor
PECAM-1 = platelet endothelial cell adhesion molecule 1

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Introduction

The current increase in the incidence of end-stage renal disease, which is mainly due to the progressive population ageing and to the epidemic spread of type 2 diabetes mellitus in industrialized countries, has prompted the development of new research aiming to establish alternative methods of renal function replacement. At present the survival of patients with terminal uremia is possible thanks to dialytic methods and renal transplant. Yet both approaches have relevant drawbacks, due to the need for suitable vascular or peritoneal access, in the former case, and for transplant organs in the latter. The ideal solution for overcoming these difficulties would be to “regenerate” the damaged organ. The adult mammal kidney can repair damage from acute injury, characterized by cellular apoptosis, through the proliferation of damaged intrarenal tissue cells1. Moreover, recent studies on mammals have suggested that bone marrow cells or renal mesenchymal cells can dedifferentiate after reaching the site of injury and contribute to the repair of the damaged nephrons2-7. However, while the repopulation of existing nephrons allows repair to occur, it is not yet possible to achieve de novo renal regeneration, since the formation of new nephrons in mammals takes place in newborns, there being an apparent exhaustion of stem cells in the adult kidney9,9. The aim of Regenerative Medicine is to develop new therapies involving the stimulation and induction in humans of regenerative processes that are already known to occur in other species, such as reptiles and fishes. The evolution of living organisms is considered a response to the environmental pathological stimuli, and the ability to recover after the loss of or reduction in a function significantly differs from species to species. The above approach, however, calls for a deep knowledge of the complex networks regulating the production of hormones and mediators involved in tissue regeneration. Findings made in recent research indicate innovative cellular and tissue engineering pathways that may restore and maintain renal function in the nephropatic patient10:

1. The so-called artificial kidney consists of an implantable filter, made using nanotechnology techniques, which can be fed with human renal cells11.
2. “Renal refurbishment” makes use of the properties of stem cells in order to repair the injured organ in situ12.
3. The “cultured kidney” technique consists of the development in vitro and implantation in vivo of a set of nephrons that purify blood and form urine. This method calls for a nephrogenic phase in vitro followed by autologous pluripotent stem cell differentiation (nuclear transfer, somatic cell reprogramming)13,14.
4. The term “renal cooptation” implies that organs other than the kidney, including the peritoneal membrane, are responsible for the excretion of toxins and other metabolic waste products. Cellular transfection in situ and/or the implantation of kidney cells would in fact allow the extra-renal development of essential solute transport and hormonal synthesis15.

The above cellular and tissue engineering strategies enable the use of complementary approaches of replacement and maintenance of renal function in patients with impaired renal function. In this context, the embryonic renal development phases represent a basic study model for obtaining information on the repair mechanisms and function of this organ.

Glomerulogenesis

The mammalian renal glomerulus, a highly developed vascular bed, acts as a filter allowing the filtration of small molecules, such as water, sugars, electrolytes and small proteins, while it represents a barrier for high molecular weight proteins and circulating cells. The correct development and maintenance of this structure throughout life is crucial to the prevention of disease16.

The glomerulus emerges from one extremity of the S-shaped body during nephron development17 (Figure 1). A stratum of columnar epithelial cells, situated at this site, is responsible for the development of visceral epithelial cells or podocytes. The basal portion of these cells rests on the future glomerular basement membrane (GBM). A cleft between the podocytes and the cells, contralateral to the basement membrane, contributes to the formation of the tubular portion of the nephron. Cells that contribute to the formation of glomerular capillaries (i.e. endothelial and mesangial cells) migrate into this gap. On the other side of this area containing future podocytes and overlying their apical surface there is a lining of thin cells which are transformed into the parietal epithelium, also known as Bowman’s capsule. Glomerular development, a dynamic process, involves the transformation of the original capillary component into a plexus
of six to eight individual loops, and the simultaneous migration of the podocytes to be distributed around them. Platelet-derived growth factor (PDGF) signalling is required for the correct assembly of the glomerular capillary loops, and mesangial cells are the main renal cell type by which PDGF exerts its effects\(^{18}\). While developing, the capillary bundle dislocates the layer of future podocytes. This layer of future podocytes, in turn, forms a “pocket” surrounding the capillary bundle which remains in contact with the external vasculature through an arterial and venous supply that will become part of the glomerular stalk. As the primitive podocytes form this pocket, the GBM continues to act as a constant barrier between the epithelial and capillary components. The podocytes do not remain a columnar epithelium: once they form the pocket, they start to lose their lateral cell-cell connections to each other, but remain attached to the GBM. During this stage, they also begin to migrate around the capillary loops, so that they no longer form a continuous uniform patch of cells. At this stage, the glomerulus is a structure distinct from the remaining nephron. This phase of glomerular development and podocyte maturation marks the start of formation of foot processes; their assembly beginning with the selective disconnection of podocytes from the GBM. Nevertheless, the appearance of mature podocytes, with foot processes extending a significant distance from the main cell body, also suggests a process by which filopodia-like cytoplasmic extensions extend in the form of scaffolding around the capillary loops\(^{19}\). The inter-digitation of podocyte foot processes around the capillaries, a distinctive feature of glomerular development, is essential to the preservation of renal function and the prevention of glomerular disease. GBM maturation is another significant step in glomerular development, a specialized basal lamina representing an important component of the protein barrier that prevents high molecular weight proteins from leaving the circulation while passing through the glomerular capillary bed. The major components of GBM are type IV collagen, laminin and the heparan sulphate, proteoglycan agrin\(^{20}\). During GBM assembly, is important the laminin-1 heterotrimer (alpha 1, beta 1 and gamma 1 chains). First expressed in vascular clefts of comma- and S-shaped bodies, is finally replaced by laminin-11 (alpha 5, beta 2 and gamma 1 chains), which persists into maturation. As demonstrated by St John et al, both endothelial cells and podocytes synthesize laminin-1 and -11 chains during glomerular development\(^{21}\). There is also a shift in type IV collagen expression; the early nephron mainly expresses subunits 1 (IV) and 2 (IV)\(^{22}\); upon maturation of the GBM, a shift to 3, 4, and 5 (IV) subunits occurring\(^{23}\). Slit diaphragm (SD) assembly, an important phase in glomerular development, leads to the formation of a structure that connects adjacent foot processes; made up of a protein complex, it forms part of the protein barrier. Nephrin, a transmembrane protein localized expressly in the renal podocytes, is an important structural component of the slit diaphragm. Mutations in this protein in humans have been associated with the Finnish form of the congenital nephrotic syndrome\(^{24,25}\). Nephrin
contains various tyrosine residues that are targets for phosphorylation by, for example, the Src family kinase FYN. At these tyrosine residues, transitory phosphorylation occurs during glomerular development, concomitant with the formation of mature foot processes. Nephrin phosphorylation triggers the recruitment of the adaptor protein NCK and cytoskeletal reorganization in podocytes. This supports the hypothesis that NCK-mediated cytoskeletal organization is related to foot process formation. In mice podocytes, Nck1 and Nck2 mutation precludes the assembly of normal foot processes. Interestingly, nephrin phosphorylation has also been observed during podocyte injury, when foot processes disassemble. This may signal the beginning of a repair process aimed at refurbishing the foot process structure. Development of the podocyte line is closely related to the differentiation and maturation of the two other major cell compartments in the glomerulus: fenestrated endothelial and mesangial cells. Podocytes produce a variety of vascular growth factors, such as VEGF-A (vascular endothelial growth factor A), VEGF-C, angiopoietin 1, and Ephrin B2, while the adjacent endothelial cells express the corresponding receptors. Podocytes begin to express all isoforms of the VEGF-A gene in S-shape bodies and continue to express them in mature glomeruli. The major signalling receptor for VEGF-A, VEGFR2 (also known as FLK1), is expressed by endothelial cells as they migrate into the vascular cleft adjacent to the podocyte precursors. VEGF-A production by podocytes is of critical importance in the formation of a functional glomerular filtration barrier and the fenestrated endothelial capillary system. Loss of the VEGF-A gene from developing podocytes in mice leads to arrested glomerular development and the absence of the glomerular endothelium.

Tubulogenesis

Two key events can be identified in the embryonic development of the renal tubule: (1) the ureteric bud (UB) extends outward from a pre-existing epithelial tube, the Wolffian duct, giving rise to the branched collecting duct system; (2) the metanephric mesenchyme (MM) at the terminal branches of the UB undergoes mesenchymal-epithelial transition, followed by tubular organization and elongation, with the formation of the proximal nephronic segments (Figure 2). Active interactions between the mesenchyme and UB appear to regulate vectorial ar-

**Figure 2.** The diagrams depicting the morphological events during mammalian kidney development. The mammalian organism forms three excretory organs, all of which are derived from the intermediate mesoderm. The first and most primitive organ, the pronephros, becomes functional in some fish although it has no obvious function in the mammalian embryo and after a short time it disappears. Longitudinal of the pronephros. The pronephros is replaced by the mesonephros, which is found in high fishes and amphibians, whereas it degenerates and is replaced by the definitive kidney, the metanephros, in mammals. Longitudinal of the mesonephros. The metanephros generates when the Wolffian duct elongates posteriorly and encounters the MM where the UB emerges. The metanephros and its generation of adult mammalian kidney is shown in a longitudinal section.
borisation, as well as the tapering and terminal differentiation of the collecting system. Completion of branching is correlated with the induction of mesenchymal and local extracellular matrix changes. Any disturbance in these mechanisms and/or single-nucleotide polymorphisms in genes regulating UB branching may cause a predisposition to various renal diseases (e.g., hypertension and chronic kidney disease) by altering the number of nephrons.

The different events in renal tubule development are mediated by several soluble factors, including positive stimulators, such as the epidermal growth factor (EGF), the transforming growth factor-\(\alpha\) (TGF-\(\alpha\)) and negative regulators, such as TGF-\(\beta\). A body of experimental evidence supports the role played by these molecular factors. Findings reported in some studies indicate that EGF plays an important role in tubule formation: EGF and EGFR are, in fact, expressed in the embryonic kidney and a defective collecting duct system has been observed in EGFR knockout mice. In the early stages, immune-reactivity to EGF and TGF-\(\alpha\) is detected in all metanephric structures and, from the seventh week onward, it is decreased in differentiating nephrons. EGF and TGF-\(\alpha\) patterns of appearance suggest that these factors play a role in the induction, proliferation and growth of metanephric structures and any disorders in this pattern may cause a reduction in kidney growth. In 1990, Taub et al demonstrated that TGF-\(\alpha\) mRNA is expressed in the kidneys (metanephros) of 9 and 10-day mouse embryos, being identified immediately prior to the branching morphogenesis of the embryonic metanephros. Its appearance at this stage is consistent with the concept that TGF-\(\alpha\) plays a role in early kidney development. Another member of the EGF family of growth factors, the heparin-binding epidermal growth factor (HB-EGF), may operate as a morphogen in renal epithelial tubulogenesis. HB-EGF is, in fact, strongly expressed in the embryonic rat kidney (embryonic days 18-20), its levels still being increased in the neonatal kidney (day 10) compared with the low basal levels observed in adult kidney. Immunohistochemical analysis confirms that immunoreactive HB-EGF expression in the fetal rat kidney is localized mainly in the UB. Moreover, many Wnt signalling components have been implicated in renal development. In Xenopus pronephric development as well as the murine metanephoi, the glycoprotein Wnt-4 has been shown to be fundamental to renal tubule formation, although little is known regarding the definitive downstream signalling pathway(s) mediating Wnt signal-effects in renal organogenesis. The inhibition of Wnt/beta-catenin signalling within the pronephric field of Xenopus induces significant losses in renal epithelial tubulogenesis. Wnt/beta-catenin signalling, required throughout the pronephric primordium, is essential for the development of proximal and distal tubules of the pronephros as well as for the development of the duct and glomus. Although less pronounced than the effects upon later pronephric tubule differentiation, the inhibition of the Wnt/beta-catenin pathway decreases the expression of early pronephric mesenchymal markers, indicating that it is also essential to early pronephric patterning. Likewise, the upstream inhibition of Wnt/beta-catenin signals in zebrafish reduces pronephric epithelial tubulogenesis. The hepatocyte growth factor (HGF) may also play a role in renal tubule formation. In 1991, Montesano et al demonstrated that HGF could induce Madine-Darby canine kidney (MDCK) cell-derived cysts to form tubular structures when suspended in a collagen matrix. TGF-\(\beta\), one of the most prominent negative regulators, has been shown to primarily inhibit HGF induced branching of the tubule, but not its elongation. Branching patterns are regulated by a precise temporal and spatial balance between branching morphogens, such as HGF, and inhibitory morphogens, such as TGF-beta superfamily members. An important role appears to be played by the extracellular matrix (ECM). Some ECM proteins, such as laminin, entactin and fibronectin, facilitate the formation of branching tubular structures and enhance their complexity. Other ECM proteins, such as Type IV collagen, heparan sulfate proteoglycan and vitronectin, markedly inhibit HGF-induced morphogenesis and TGF-beta modulates both tubulogenesis and branching. Finally, a tubulogenic morphogen, such as HGF and a tubulogenesis-inhibitory morphogen, such as TGF-beta, can, in the context of the dynamic matrix present during epithelial tissue development, modulate the degree of tubule (or duct) formation, as well the length of these tubules, and the extent of their arborisation. Other members of TGF-\(\beta\) family, such as bone morphogenetic proteins (BMP), can also modulate in vitro tubulogenesis in mouse renal collecting duct cells. BMP2 and high doses of BMP7 markedly inhibit tubule formation, while low doses of BMP7 appear to stimulate tubulogenesis. The tubulogenic growth factor/receptor combinations activate a series of cytosolic and
membrane associated signalling events that modulate cell shape change, division and motility, playing a role in the development of tubule both in vitro and in vivo. However, the exact subcellular mechanisms involved in this process are only beginning to be understood (Figure 3). Repair of the injured adult kidney appears to involve a similar process of cellular differentiation and organization into a functional tubular epithelium.

Renal Tissue Repair

Such cells have been identified in the adult rat kidney as epithelial label-retaining tubular cells (LRTC); their active proliferation has been found to contribute to tubular regeneration in an ischemic/reperfusion rat model. Further studies by the same group focused on the behaviour of LRTC in following unilateral ureteral obstruction (UUO). In response to this injury, LRTC were present not only in tubules but also in the interstitium of the UUO kidney. An increase in the number of LRTC, a change in their regional distribution and the expression of fibroblast markers suggested that, in this model, the LRTC population could proliferate, migrate and transdifferentiate, also potentially contributing to renal fibrosis. Further characterization was carried out by Maeshima et al on isolated Hoechst low/LRTC using FACS, based on reduced Hoechst signal due to bromodeoxyuridine (BrdU) incorporation. These cells demonstrated a phenotypic plasticity, a tubulogenic capacity and the capacity to integrate in the developing kidney.

Bussolati et al were the first to describe the isolation and characterization of potential progenitors from adult human kidney using specific surface markers.

In human adult kidneys, a subset of parietal epithelial cells localized at the urinary pole of the Bowman’s capsule was identified, based on CD24 and CD133 co-expression. These cells show self-renewal and multi-differentiation potential and, when isolated and injected into mice with acute renal failure from glycerol-induced rhabdomyolysis, regenerate dif-

Figure 3. Scheme of a mature nephron and its developmental origin: the glomerulus, proximal tubule, Henle’s loop, distal tubule and connecting tubule derive from the metanephric mesenchyme (MM), whereas the collecting ducts derive from the ureteric bud (UB).
different portions of the nephron, reduce tissue necrosis and fibrosis and significantly improve renal function. No tumorigenic potential was observed. Then, CD24+CD133+ cells represent a compartment of multipotent embryonic progenitors that persist in human kidneys from early stages of nephrogenesis. Their ability to repair renal damage, together with their apparent lack of tumorigenicity, suggests the future prospect of using them in the treatment of renal failure.

**Stem Cells and Kidney Repair**

Bone marrow (BM) contains at least two populations of stem cells: hematopoietic stem (HSCs) and mesenchymal stromal (MSCs) cells, which provide stromal support for HSCs. It also contains many other hematopoietic cell types involved in immune surveillance, inflammatory responses and pathogen removal. It has long been believed that bone marrow, a known source of stem cells, may contribute to the repair of other organs.

Duffield et al. have pointed out that the contribution of bone marrow-derived cells to renal repair is relatively low (0.06-8%). Furthermore, Held et al. have shown that cell infusion of 20 to 50% can be induced between bone marrow-derived cells and renal tubular cells under conditions of chronic renal damage. Nevertheless, BM transplantation can improve renal function.

As demonstrated by Rae et al., resident monocytes are present in the developing kidney prior to the start of nephrogenesis, and the macrophage colony-stimulating factor (CSF-1) is able to increase this population and simultaneously increase the rate of renal development. This resident macrophage population may also play a role in organ homeostasis and response to injury.

Numerous studies have been conducted to ascertain whether the reparative capacity of the kidney is enhanced by MSC, another widely cited candidate for the ability of the BM cells to ameliorate renal damage. One relevant advantage of using MSCs for renal repair is their ability to “home” to the injured kidney. Herrera et al. found that the increased expression of hyaluronic acid in the injured kidney was responsible for MSC migration, since these cells express the receptor for HA (hyaluronan), CD44.

MSCs isolated from mice without CD44 failed to reach the injured kidneys and provided no protection from injury. MSCs are immune-privileged, in that they obviate allogenic rejection in humans by failing to induce a proliferative T-cell response. Coupled with their immunomodulatory advantage (although potentially less effective *in vivo* than *in vitro*), this immune-privileged status raises the possibility of an ‘off-the-shelf’ cellular product appropriate for any recipient. Since MSCs can also be obtained from autologous sources, including renal patients, they are ideal vehicles for the delivery of others genes known to be conducive to kidney repair.

In their recent paper, Hagiwara et al. described MSCs over-expressing human tissue kallikrein, a protein they had previously shown to protect the kidney from damage. These modified MSCs provided greater protection than unmodified MSCs.

A recent study has shown that human amniotic-fluid derived stem cells can form early nephron structures (renal vesicles, S- and comma-shaped bodies) when injected into embryonic mouse kidneys. If these cells were used, some of the ethical problems associated with embryonic stem cells would be avoided. However, other obstacles, linked to the delivery of these stem cells and the immune rejection of allogenic stem cell sources, have yet to be overcome.

**Endogenous Stem Cells**

Numerous organs, particularly those with a high cellular turnover, are believed to harbor a stem cell population that sustains normal organ structure and contributes to repair. This applies to the skin, intestine, stomach and hematopoietic system and, probably, the brain. For other organs, including the kidney, the existence of multipotent stem cells is still under debate. Currently, it is not believed that the adult kidney can undergo true regeneration, because it is regarded as a highly terminally differentiated organ, but it is known to have a considerable capacity for the morphological restoration of tubules and the recovery of function following injury. Toxic and ischemic insults to the kidney lead to acute renal failure, most often manifest as acute tubular necrosis. After injury, the kidney undergoes a regenerative response that induces recovery of renal function. New cells are required to replace damaged cells. Three possible sources of new tubular cells are: adjacent, less damaged tubular cells; extrarenal cells, in all probability coming from bone marrow, which home to the injured kidney; resident renal stem cells. Stem cells exist in the MM and can give rise to all of the cell types of the adult kidney, except those that are derived from UB.

Renal stem cells persist in the adult kidneys of other organisms, such as the skate and the fresh
water teleost. These cells can take part in new nephron formation after partial nephrectomy.

Potential candidate stem cells have been identified in the adult mammalian kidney using different identification methods, and this experimental evidence could contribute to the future development of cell therapy for renal regeneration.

Given the lack of definitive evidence for an endogenous renal stem cell and the apparent absence of embryonic progenitors after the cessation of nephrogenesis, it is important to consider whether it is feasible to employ induced reprogramming to facilitate either renal repair or regeneration. In the kidney, a number of factors have been shown to cause epithelial-mesenchymal transdifferentiation (EMT) of adult tubular epithelial cells in vitro, although the underlying mechanisms. For example, TGFβ1 signalling, are mainly associated with fibrogenesis and disease.

Finally, a recent microarray profiling experiment has demonstrated that the majority of genes that are co-ordinately regulated during dedifferentiation in Amoebia are also up-regulated during its development.

While the location of putative stem/progenitor cells has been described in some studies, the isolation of stem/progenitor activity based upon location is less common. In a report by Kitamura et al., isolation of potential renal stem/progenitor cells was performed based on sub-compartmental dissection of the adult rat kidney.

The cells expressed mature renal epithelial markers, including AQP-1, AQP-2 and CLC-K, thus making this proposed progenitor line very similar to that of differentiated epithelial cells.

**The Renal Blastema and The Neonephrons**

While no evidence is available to demonstrate the presence of a residual nephrogenic zone in the post-natal human kidney, reversion to a nephrogenic zone is still possible by means of the reprogramming of terminally differentiated adult kidney cells. There is ample evidence that renal epithelial cells can be reprogrammed to trans-differentiate through an EMT event in vitro, and this is supported by observations made in vivo in the course of renal disease. TGFβ1 is a potent factor for inducing EMT in kidney cells. If renal epithelial cells can be reprogrammed to undergo EMT to form a cap mesenchyme-like population of renal progenitors, this may represent a possible strategy for inducing re-growth in order to obtain new nephrons. Numerous transcription factors are involved in the specialization of the mesenchyme progenitor population in the developing kidney. The individual inactivation of Wt1, Pax2, Eya1, Six1, Six2, Osrl and Sall1 all result in renal agenesis. The fact that other Wnt family members will suffice to achieve agenesis in vitro suggests that the recapitulation of the exact development signal may not always be required. However, in the absence of a source of Wnt9b and a viable connection to a patent collecting duct system and surrounding vasculature, no neonephrons will function. During its development, the MM itself is involved in inducing the UB to arise from the mesonephric duct and grow towards the MM. Factors such as the glial cell line-derived neurotrophic factor (GDNF) and vascular endothelial growth factor (VEGF) are of critical importance, as they have been shown to induce the formation of the UB (GDNF) and vascular tissue (VEGF) in vitro. Such factors may allow for the re-initiation of collecting duct branching in the adult kidney. Lateral branching from the ureteric stalk after the removal of the tips of the embryonic UB has been shown to be feasible, at least within a specific embryonic window in the presence of an early renal blastema. Complex as this process may seem, it may prove more effective in the treatment of chronic renal disease than the humoral effects of MSCs or endogenous proliferation and re-epithelialization. In their recent study, Rosines et al. determined the culture conditions required for the differentiation in vitro of a Wolffian duct pre-emptively isolated from its mesodermic stroma. For its development, the Wolffian duct requires different growth factors to be added to the culture medium, such as the glial cell-derived neutrophic factor (GDNF) and the fibroblast growth factor (FGF) types 1 and 7. The thus-induced UB morphogenesis reproduces the naturally observed T-shape well and each of the two parts of the T maintains the ability to undergo subsequent development. The UB maintains its ability to induce a mesenchymal-epithelial transition in contact with a rat embryo isolated MM. Tubulogenesis and the continuity between the two structures at the collector channels lead to the formation, in vitro, of complete and functioning nephrons.

The renal tissue created in vitro rapidly develops a neo-vascularization and its renal subcapsular implantation, showing glomerulogenesis signs and the expression of an endothelial marker: the
platelet endothelial cell adhesion molecule 1 (PECAM-1). The main limitation of these strategies for achieving kidney neo-formation depends on the need to isolate the primitive nephrogenic structures, rich in pluripotent stem cells, from an embryo. By reproducing renal organogenesis in vitro, from embryonic, amniotic or adult cells, it would be possible to obviate this technical and ethical limitation, and transfection could enhance immune-compatibility and functionality. Moreover, new cellular xenotransplantation strategies might be developed using embryonic cells known to be less immunogenic.

Conclusions

Regeneration has long been a deeply fascinating issue that has inspired several myths. At the most, it holds the promise of immortality and rejuvenation and, at the least, of the reconstruction of injured organs. Among vertebrates, only teleost fishes and urodele amphibians have kept their lifelong natural ability to regenerate injured or amputated parts. The recently gained understanding of the regeneration mechanisms of complex structures in the adult salamander has opened original lines of research. Regenerative Medicine, a new medical domain, tries to develop therapeutic pathways through the stimulation of natural regenerative processes also in humans. The mechanisms for functional recovery are now under the scrutiny of researchers aiming to further their understanding of the natural network that regulates the regeneration process and the intricate connections that lead to the production of hormones and mediators fundamental to the regeneration of different tissues. The development of these lines of research, also in nephrology, may yield new therapeutic strategies designed to enhance the intrinsic regenerative capacities of the kidney.

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