Directed Differentiation of Pluripotent Stem Cells to Kidney Cells

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Summary: Regenerative medicine affords a promising therapeutic strategy for the treatment of patients with chronic kidney disease. Nephron progenitor cell populations exist only during embryonic kidney development. Understanding the mechanisms by which these populations arise and differentiate is integral to the challenge of generating new nephrons for therapeutic purposes. Pluripotent stem cells (PSCs), comprising embryonic stem cells, and induced pluripotent stem cells (iPSCs) derived from adults, have the potential to generate functional kidney cells and tissue. Studies in mouse and human PSCs have identified specific approaches to the addition of growth factors, including Wnt and fibroblast growth factor, that can induce PSC differentiation into cells with phenotypic characteristics of nephron progenitor populations with the capacity to form kidney-like structures. Although significant progress has been made, further studies are necessary to confirm the production of functional kidney cells and to promote their three-dimensional organization into bona fide kidney tissue. Human PSCs have been generated from patients with kidney diseases, including polycystic kidney disease, Alport syndrome, and Wilms tumor, and may be used to better understand phenotypic consequences of naturally occurring genetic mutations and to conduct "clinical trials in a dish". The capability to generate human kidney cells from PSCs has significant translational applications, including the bioengineering of functional kidney tissue, use in drug development to test compounds for efficacy and toxicity, and in vitro disease modeling.

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Approximately 2,000,000 people worldwide suffer from end-stage renal disease. Existing strategies for renal replacement therapy include dialysis and transplantation. Although these treatment options can prolong survival, both have serious limitations. Dialysis is associated with significant morbidity, and mortality rates are 6.5 to 7.9 times greater than the general population. Although dialysis functions to remove waste products and help maintain electrolyte, acid-base, and fluid balance, it cannot substitute all of the kidneys' functions. Kidneys are the most frequently transplanted organs, but demand greatly outweighs supply and the waiting period is typically several years. Acute rejection occurs in approximately 15% of transplant cases, and successfully transplanted kidneys require life-long immunosuppression to reduce the rate of chronic rejection.[1] For these reasons, kidney regeneration from human stem cells, ideally patient immunocompatible, is an attractive solution for renal replacement.

FEATURES OF NEPHROGENIC STEM CELLS

The adult mammalian kidney lacks the capacity to generate entirely new nephrons or to regenerate segments from resected nephrons. Nephrogenesis ceases before or shortly after birth in human beings and mice, concomitant with the disappearance of the nephron progenitor pool.[2–4] Nevertheless, adult kidney epithelial cells are capable of extensive dedifferentiation, proliferation, and repopulation of damaged tubules after injury.[5,6] These processes make it possible for existing nephrons to recover after acute kidney injury but do not provide a source for new nephrons. Clinically, the ramifications of this are that human beings are born with a limited number of nephrons, which can be damaged irreversibly. The loss of a significant number of nephrons is therefore an event with important long-term consequences.

Because embryonic kidney cells are the only cells capable of forming new nephrons, derivation of such cells is likely to be required to create new nephrons for patients with kidney disease. Understanding nephron development in the embryo therefore is essential to designing and interpreting experiments that aim to generate new nephrons in the adult. Mammalian kidney development has been summarized elsewhere in greater detail.[3,7] The pronephric duct arises from the intermediate mesoderm (IM) of the embryo at approximately day 22 of gestation in human beings, or day

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8 in mice. The duct iteratively invades the adjacent mesenchyme, giving rise sequentially to the pronephros, mesonephros, and, finally, the metanephros, which in amniotes will develop into the adult kidney. Formation of the complete nephron tubule involves reciprocal interactions between two tissues, the ureteric bud (UB) and the metanephric mesenchyme (MM).[8] Molecular analysis over the past 60 years has identified a variety of signaling molecules that specify these tissues (including Wnts, fibroblast growth factors [FGFs], bone morphogenetic proteins [BMPs], glial cell–derived neurotrophic factor, and hepatocyte growth factor [HGF]), as well as transcription factors (WT1, SIX1, SIX2, PAX2, PAX8, and HOXD11) that regulate branching morphogenesis and maintain the nephron progenitor cell pool.[3,7,9] In addition to the tubular components, the patterned kidney also includes a significant population of vascular and interstitial cells in a complex three-dimensional (3D) configuration.

Decades of experiments on isolated metanephroi have established a framework for understanding the characteristics of these cells and their potential for regeneration. When cultured together ex vivo, UB and MM can undergo branching nephrogenesis. This ability to self-organize makes it feasible to envision nephron regeneration from these embryonic cell types. When implanted into living hosts, fetal kidney cells isolated from various mammals, including human beings, have been reported to form new vascularized nephrons that can produce urine and prolong survival after nephrectomy.[10–13] Notably, the ability of metanephric tissue to undergo branching nephrogenesis requires both the UB and the MM; when separated from each other, the UB does not branch, and the MM does not survive unless it is co-cultured with appropriate inducing tissues such as embryonic spinal cord.[14] Various survival and differentiation factors have been identified for metanephric cells, notably BMPs and FGFs, although long-term cultivation of these cells in vitro remains a challenge for the field.[3,15,16]

PLURIPOTENT STEM CELLS

Pluripotent stem cells (PSCs) are cultured populations of early embryonic progenitors, believed to represent blastocyst or epiblast cell types from which the entire soma is derived. They are defined by two characteristics: pluripotency, the ability to give rise to diverse and complex tissues from each of the three embryonic germ layers; and self-renewal, the capacity to proliferate and expand indefinitely in culture without transformation. Because adult mammalian kidney cells cannot regenerate new nephrons,[5] and fetal kidney cells undergo apoptosis or differentiate in culture,[3,16] PSCs are currently the only long-term cultivatable cell type capable of neonephrogenesis. The most convincing demonstration of this has been in cloned mice, in which the entire soma, including the kidneys, can be derived from cultured PSCs via tetraploid complementation.[17,18]

PSCs include both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). ESCs are grown in primary culture after derivation from embryos, whereas iPSCs are cells derived from the adult that transcriptionally have been reprogrammed to an ESC-like state. iPSCs greatly resemble ESCs, although some differences may exist between the two cell types.[19] Patient-derived iPSCs have dual value as a laboratory resource for studying the cellular basis of human disease and as a potential source of immunocompatible replacement tissue.[20–23] Because they are pluripotent and self-renewing, iPSCs provide a practically unlimited opportunity to obtain diverse cell types and tissues with naturally occurring human disease mutations for experimental investigation. This constitutes a great advantage over many primary cell types in culture, which are difficult to obtain and often senesce or dedifferentiate rapidly.[21] Studies in mice show that somatic cells derived from iPSCs are immunocompatible with the hosts from which they derive.[24] In theory, iPSCs could be used to generate tissues or organs that would be immunocompatible with the individual from whom they were derived. These characteristics were considered so significant that the discovery of iPSCs was co-awarded the Nobel Prize in Medicine in 2012, only 6 years after its initial publication.[25] Although the application of iPSCs to kidney research is still in its infancy, recent progress has suggested utility for both disease modeling[26] and regenerative[27–31] approaches.

METHODS OF DIFFERENTIATION OF PSCs

By using biochemical treatment regimens, PSCs have been differentiated successfully into various types of cells and tissues, including hepatic, neural, hematologic, pancreatic, and cardiac lineages. The various approaches have been reviewed elsewhere.[32] When PSCs are dissociated and deprived of growth factors that sustain pluripotency (FGFs and activin in human PSCs under standard growth conditions, or leukemia-inhibitory factor in mouse PSCs), they undergo stochastic differentiation into embryoid bodies (EBs) in vitro or teratomas in vivo.[21,33] These 3D growths include diverse somatic cell types and tissues representative of the three embryonic germ layers and can be used as an indicator of pluripotency of the PSCs. Histologic and immunohistochemical examination of these stochastic growths suggests the possibility that PSCs can differentiate stochastically into kidney cells