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Generating Kidney Organoids from Human Induced Pluripotent Stem Cells: Potential for Treating Kidney Disease

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ABSTRACT

An estimated 1 million people worldwide die each year from untreated kidney disease, which is a major public health issue. Most of them require maintenance dialysis or renal transplantation. Dialysis is not a successful treatment option due to high medical expenditures and negative effects on the patients. Kidney transplantation is limited by the shortage of organ donors and the possibility of immune rejection. Kidney organoids derived from human pluripotent stem cells have emerged as an attractive source for regenerative medicine and disease modeling for *in vitro* models of kidney development, physiology, and disease. The usefulness of such cells in acute and chronic kidney disease has been demonstrated. This review focuses on the current literature describing the reprogramming of somatic cells to pluripotent stem cells and their differentiation into various kidney lineages.

Abbreviations: AKI: Acute Kidney Injuries; CKD: Chronic Kidney Disorders; ESRD: End-Stage Renal Disease; PCT: Proximal Convoluted Tubule; DCT: Distal Convoluted Tubule; IM: Intermediate Mesoderm; UB: Ureteric Bud; MM: Metanephric Mesenchyme; NPC: Nephron Progenitor Cells; GDNF: Growth Factor Cell Line-Derived Neurotrophic Factor; PTA: Pretubular Aggregates; CLS: Capillary Loop-Stage; RV: Renal Vesicles; CSB: Comma-Shaped Bodies; SSB: S-Shaped Bodies; ESCs: Embryonic Stem Cells; iPSCs: Induced Pluripotent Stem Cells

Introduction

Kidney disorders have emerged as a major public health concern worldwide. One in nine Americans and more than 10% of the world's population suffer from kidney disease [1]. Acute kidney injuries (AKI) and chronic kidney disorders (CKD) are major causes of morbidity and mortality worldwide [2]. The functional units of the kidney, known as nephrons, have a considerable capacity for regeneration, but damage brought on by a variety of conditions, such as nephrotoxic substances, ischemia, infections, immune-mediated tubulo-interstitial nephritis, and auto-immune illness, can exceed this capacity and result in a permanent loss of nephrons. Rapid renal function loss, severe renal inflammation, and resident cell programmed death are all characteristics of AKI. The kidney's ability for repair may be exceeded by repeated AKI incidents and more subtly harmful long-term effects from diabetes, cardiovascular disease, and hypertension [3] may cause maladaptive repair, which can lead to chronic kidney disease (CKD) [4]. There aren't many efficient and successful treatments for end-stage renal disease (ESRD), which can be caused by both CKD and AKI. ESRD significantly reduces patients' quality of life and life expectancy [5]. Patients with ESRD require treatment with dialysis or transplantation to survive. The estimated annual cost of ESRD in the USA is 120 billion dollars [6]. Transplanting a kidney from a healthy, immune-matched donor is the best course of treatment. But organ rejection and a scarcity of organ donors severely limit it.

The patient needs to take strong immunosuppressive drugs to avoid transplant rejection. Fundamental and translational studies have been carried out in the areas of kidney physiology, disease modeling, high-throughput drug efficacy and toxicity testing, regenerative medicine, and the production of functional kidney tissues for the development of a new human kidney to address these burdens. Significant advances have been made in recent years in creating kidney organoids from human somatic cells. In this review, embryonic development of human kidney will be examined first. The sources of stem cells for kidney organoids will then be covered. Finally, a review of different kidney organoids protocols will be given.

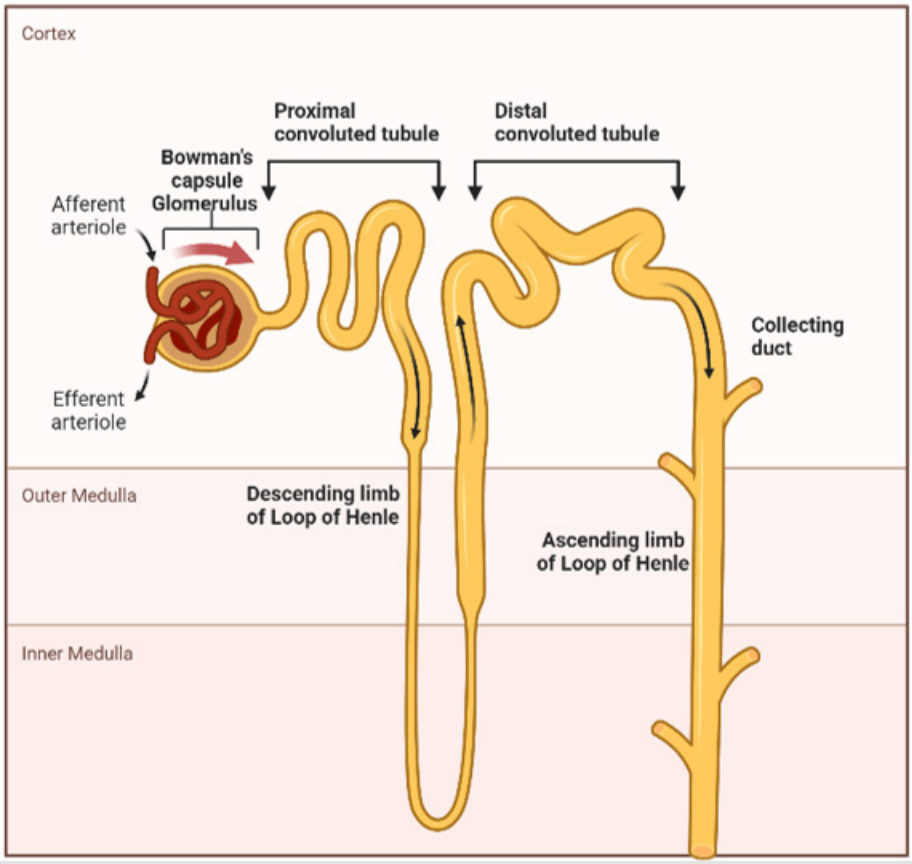

Figure 1: Parts of a nephron.

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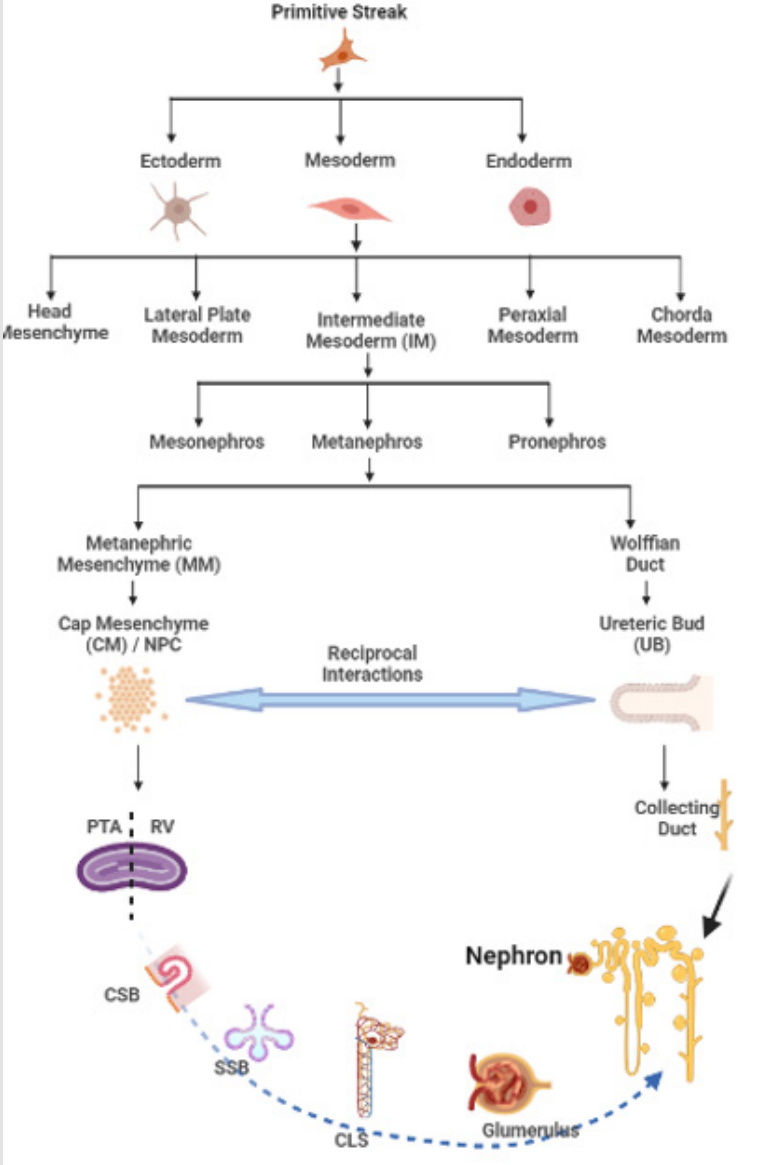

Figure 2: Embryonic kidney development.

Anatomic Description of Human Kidney Development

To establish a variety of cell differentiation approaches, it is crucial to comprehend the normal kidney development phases. In recent years, a great deal of research has been carried out to understand kidney formation and nephrogenesis [7-9]. About 25 different cell types make up the nephron, which is divided into five main segments, each of which has distinct roles Figure 1. Renal corpuscles and renal tubules are the two basic structural components of a nephron. The renal corpuscle comprises the glomerulus, a cluster of capillaries, enveloped by the Bowman's capsule, a double-layered epithelial cup. Meanwhile, the renal tubule is comprised of three main sections: the proximal convoluted tubule (PCT), the loop of Henle, and the distal convoluted tubule (DCT). Nephron development unfolds across a four-week gestational period, yielding an average of 1 million nephrons per kidney. At maturity, each kidney houses thousands of interconnected nephrons that link to an extensively branched collecting duct system. [10]. (Figure 2) illustrates the nephrogenesis process schematically. On day 15 of human development, a temporary structure called the primitive streak forms, signaling the beginning of gastrulation [11]. The primitive streak emerges as an elongated groove on the epiblast, outlining the anterior-posterior and medial-lateral axes in the evolving embryo. The presence of the primitive streak establishes bilateral symmetry and initiates germ layer formation.

During this course, the embryo develops from a single layer of cells to three layers of embryonic germ cells: the endoderm, ectoderm, and mesoderm [12]. Later, these layers mature into specific body systems. In a neurula stage embryo, the chorda mesoderm, paraxial mesoderm, intermediate mesoderm (IM), lateral plate mesoderm, and head mesenchyme are the five areas that make up the mesoderm. The cells in the primitive streak's anterior region develop into paraxial mesoderm. The lateral plate mesoderm develops from the primitive streak's posterior cells. IM germ layers are positioned between the paraxial and lateral plate mesoderm. The IM is where the kidneys and ureters grow in all vertebrates. Three sets of tubular nephric structures that develop from IM are the pronephros, mesonephros, and metanephros. The metanephros develops into a functioning kidney throughout embryonic development. The Wolffian duct originates from the mesonephros and sprouts into the ureteric bud (UB) [13]. Metanephric mesenchyme (MM) forms because of posterior IM cell differentiation [14]. MMs contain nephron progenitor cells (NPC). The MM and UB interact in a complicated series of reciprocal interactions leading to the development of the kidney. The MM secretes glial growth factor cell line-derived neurotrophic factor (GDNF) that stimulates the Wolffian duct to produce UBs. The UB tip branches serially to generate the collecting duct system, whereas the MM forms nephrons [15]. GDNF is continuously produced by NPCs to allow the reciprocal interactions MMs and UBs.

To keep the NPC in an immature stage, SIX2 expression must be present [16]. Near the UB branch tips, MM cells condense to form pretubular aggregates (PTA) that go through a mesenchymal-epithelial transition to produce renal vesicles (RV). The renal vesicles develop into capillary loop-stage (CLS) nephrons via comma-shaped bodies (CSB), and S-shaped bodies (SSB). At the SSB stage, cells from the distal nephron segment start infiltrating the collecting duct epithelium, establishing the connection between the nephron and the collecting duct. The CLS is then started when endothelial cells begin to penetrate the proximal section of the SSB. This stage is also marked by the vascular system's development, including the glomerular capillaries, arteries, and veins, as well as the emergence of the early Henle loop [17,18]. Collecting ducts enlarge after ureteric tree branching stops in the middle of pregnancy, and new nephrons develop along each duct's course. After 34 to 36 weeks of pregnancy, no more nephrons develop in humans.

Sources of Stem Cells for Kidney Organoids

Embryonic stem cells (ESCs) Figure 3 can transform or differentiate into different embryonic germ lineage: ectoderm, mesoderm, and endoderm [19]. ESCs have infinite self-regenerative abilities. Mouse embryonic stem cells were used to investigate early differentiation protocols toward the kidney lineage and to uncover several growth factors that were effective inducers of kidney lineage cells [20]. Since then, research interest has shifted to using human stem cells to differentiate into kidney cells. There are many types of stem cells, and some work better than others in certain situations. Some stem cells have been found in the adult kidney, but they are limited. They are considered dormant unless minor repairs are required. The best-known source of these cells is the blastocyst-stage human embryo. Human embryonic stem cells are isolated from early-stage embryos that contain only about 150 cells. Since ESCs are isolated early in development, they naturally form all the organs and tissues found in adult animals [21]. Although the application of ESCs in regenerative medicine seems promising, the use of ESCs in cell therapy is inherently difficult. The use of ESCs for cell therapy is limited as a very small fraction of the total population stores their own ESCs. Moreover, clinical application of ESCs generated from embryos is related to issues such as human embryo manipulation, risk of tumorigenesis, and immunological rejection. Currently, ESCs are isolated from early embryos, so this study only has an ethical license in some countries.

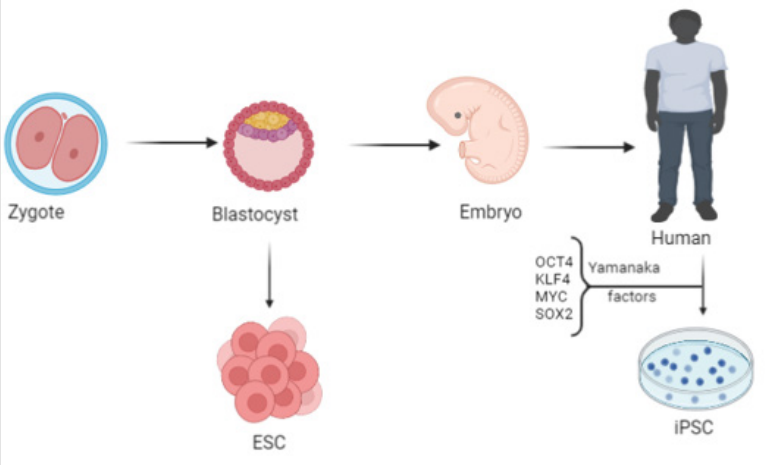

Figure 3: Embryonic stem cell (ESC) and induced pluripotent stem cell (iPSC).

After transplantation into immunocompromised mice, studies have shown that ESCs normally form tumors [22]. Recent advances have enabled the formation of kidney organoids from induced pluripotent stem cells (iPSCs) via directed differentiation. Pluripotency is the ability of a stem cell to differentiate into any of the three germ lineages that give rise to major cells of any tissue type. Many of the regenerative properties of ESCs are shared by iPSCs. A breakthrough in tissue regeneration has been achieved with the identification of four genes, OCT4, KLF4, MYC and SOX2, known as the Yamanaka factors [23], which can reprogram somatic cells into iPSCs. This implies that patients can use some of their own cells, like skin or blood cells, to create iPSCs, which can then be used to create the specific kind of cells they want. As a result, iPSCs facilitate the creation of disease models that are tailored to individual patients and immuno-compatible transplantation. Since iPSCs are immature cells and can transform into virtually any cell type in the body, an important challenge for scientists is how to differentiate these iPSCs in a coordinated manner to produce kidney organoids.

Generation of Kidney Organoids from Induced-Pluripotent Stem Cells

MM and UB exchange signals mutually to create, maintain, and complete kidney formation. However, scientists were unaware of the mechanisms underpinning how the mesoderm develops into IM and how the MM and UB lineages separate from one another. It was also unclear how the anterior posterior axis along the IM evolved to give rise to the posteriorly located metanephros. In 2014, Taguchi et al. unexpectedly observed that T+ MM precursors are developmentally separate from Osr1+ UB progenitors, which led to a significant advance in our understanding of kidney formation. They made the startling discovery that MM and UB are generated from the anterior and posterior IM, respectively, as well as the expression of distinctive markers. With this insight, researchers have developed differential protocols to develop kidney organoids from iPSCs by adjusting the exposure period, dosage, and presence of important growth factors. (Table 1) lists kidney growth factors involved in differentiation of different renal cells. Innovating studies by several research groups described protocols for differentiating iPSCs into kidney organoids [24-47]. These studies showed that the interaction between UB and MM, a prerequisite for nephrogenesis, is primarily determined by three growth factors: Wnt11, Wnt9b, and FGF9. *In vitro*, they manipulated these growth factors to control MM and UB populations. Wnt activation was controlled by CHIR99021, regulating the dominance of MM and UB populations, and FGF9 enabled MM patterning.

Takasato, et al. [8,34] reported the formation of complex 3D multicellular kidney organoids containing up to 500 individual nephrons. Transcriptional factor expression suggested that these progenitor cells developed into distinct nephron cell types, such as podocytes, proximal tubule cells, ascending limb of the loop of Henle, and distal nephrons. The same combination of growth factors aided in the expansion of endothelial vascular cells, some of which developed into glomeruli, which are crucial for filtration. Also visible were stromal cells, which are found in the space between nephrons.

Table 1: Growth factors for kidney cells.

Growth Factor	Involved in
Retinoic acid	Cell differentiation UB development
Activin A	Cell growth/proliferation Mesodermal cell differentiation
BMP4, BMP7	Renal system segmentation Anterior/posterior axis determination Mesodermal cell fate NPC differentiation Glomerular development IM cell differentiation UB/Collecting duct/Ureter development
WNT4	UB branching Epithelial to mesenchymal transition Metanephric mesenchymal cell differentiation Metanephric tubule formation Renal vesicle formation
FGF9	Regulation of mesenchymal cell proliferation Regulation of WNT Regulation of Activin
FGF5	Fibroblast growth Glial cell differentiation
GDNF	UB formation Mesenchymal to epithelial transition

Conclusion

The enhanced understanding of kidney development and kidney organoids created from iPSCs paves the door for future research focusing on the treatment of kidney disorders. In situations of AKI and CKD disease, recent developments in cell-based therapy using iPSCs have shown tremendous promise for restoring normal kidney functioning [30,46-48]. Organoids produced from iPSC hold promise to enable thorough research into the physiology of renal development and screening for consequences of brand-new or existing medications or teratogenesis. In biobanks with organoids from numerous individuals, drug efficacy testing can be done to find a general treatment as well as to deliver individualized medicine [49]. Creating kidneys from induced pluripotent stem cells (iPSCs) derived from individual patients could offer a potential reservoir of autologous organs that align with the patient's immune system, ensuring compatibility. Long-term studies are needed to create a fully functioning kidney with filtration.

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