Am J Transl Res 2019;11(2):1102-1115
www.ajtr.org /ISSN:1943-8141/AJTR0081502

Review Article

Engineering renal epithelial cells: programming and directed differentiation towards glomerular podocyte's progenitor and mature podocyte

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Received June 20, 2018; Accepted December 26, 2018; Epub February 15, 2019; Published February 28, 2019

Abstract: Current knowledge of normal developmental physiology and identification of specific cell types of the kidney at molecular levels enables us to generate various cells of the kidney. The generation of renal specialized cells in vitro with its correct molecular and functional implications is the urgent need for cellular therapy in chronic kidney diseases and for organ formation. Glomerular podocytes are one of the major renal cells lose its functionality to maintain glomerular blood filtration function. In vitro, many inductions or reprogramming methods have been established for podocytes development. In these methods transcription factors, small molecules, and growth factors play the major role to remodel stem cells into podocyte progenitors and towards mature podocytes. Micro ribonucleic acids (miRNAs) have been utilizing as another strategy to generate podocyte. In this review, current protocols for in vitro glomerular podocyte differentiation have summarized emphasizing programming methods, signaling modulation, and cytoskeletal changes. Novel ideas are also pointed out, which are required for efficient optimal glomerular podocyte generation and their functional characterization in vitro with nanoarchitecture impression of the glomerular basement membrane.

Keywords: Glomerular podocytes, differentiation, transcription factors, small molecules, growth factors, signalling, miRNA

Introduction

Kidneys are one of the vital organs for normal homeostasis of the body. Chronic kidney diseases (CKD), irrespective to their primary cause culminate in proteinuria and complete loss of kidney functions to which renal replacement therapy (RRT) is required. The current need is to replace non-functional cells from healthy cells, either providing nephron progenitors (NPs) population or to directly replace adult functional cell types.

Glomerular podocytes are one of the major renal cells associated with many renal diseases by the loss of podocytes or its function, which is to maintain glomerular blood filtration. It results in proteinuric states due to the flattening or effacement of the foot processes of podocyte. The podocytopathies include minimal change nephropathy (MCN), focal segmental glomerulosclerosis (FSGS), diffuse mesangial sclerosis, and collapsing glomerulopathy (CG). The glomerular podocytes are very specialized but incomplete epithelial cells as it demonstrates both epithelial and mesenchymal features. It carries cell polarity, low invasive capacity, anchorage dependence, and tight junction modification of epithelial features while spindle-shaped, podocyte cadherin (P-CDH) expression, neuronal cadherin (N-CDH) expression, high cell-matrix interactions, and high migration capacity of mesenchymal features [1]. Podocytes are terminally differentiated post-mitotic cells that cannot enter cell division or proliferate [2]. It expresses cyclin-dependent kinase inhibitors p27 and p57 while do not express cyclin A, Cyclin D, and Ki-67, the markers of proliferation [3]. Morphological differences of podocyte were observed during proteinuria associated nephritic diseases. In the kidney, glomerular podocyte consists of a cell

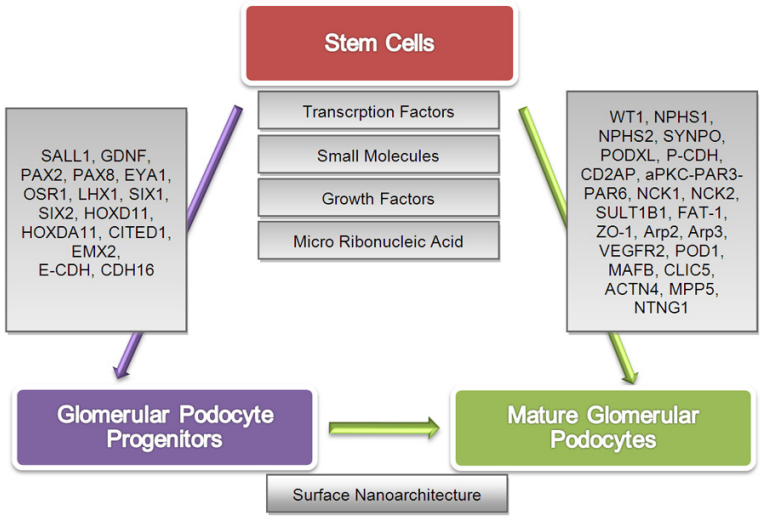

Figure 1. Stem cells can generate glomerular podocyte progenitors and mature glomerular podocytes in vitro. Stem cells are sensitive to environmental cues in culture conditions and affected by specific transcription factors, small molecules, growth factors, and micro ribonucleic acid individually or in combination to generate glomerular podocytes. They expressed protein markers that signify their status of being the glomerular podocyte progenitor or fully functional glomerular podocyte. Podocyte progenitor cells expressed nephron progenitor markers in combination with specific podocyte markers. Podocyte differentiation influenced by surface nanoarchitecture through respective gene expression (grey boxes on the arrows) in podocyte progenitors and mature podocytes. [SALL1, Spalt like transcription factor 1; GDNF, Glial derived nerve growth factor; PAX2, Paired box 2; PAX8, Paired box 8; EYA1, Eyes absent (Drosophila) Homolog 1; OSR1, Odd-skipped related 1; LHX1, LIM homeobox protein 1; SIX1, Sine oculis homeobox homolog 1; SIX2, Sine oculis homeobox homolog 2; HOXD11, Homeobox D11; HOXDA11, Homeobox DA11; CITED1, Cbp/P300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 1; EMX2, Empty spiracles homeobox 2; E-CDH, Epithelial cadherin; CDH16, Cadherin 16; WT1, William's tumor 1; NPHS1, Nephrin; NPHS2, Podocin; SYNPO, Synaptopodin; PODXL, Podocalyxin-like protein 1; P-CDH, Podocyte cadherin; CD2AP, CD2-associated protein; aPKC-PAR3-PAR6, Atypical protein kinase C-Partitioning defective 3 homolog-Partitioning defective 6 homolog complex; NCK1, Non-catalytic region of tyrosine kinase adaptor protein 1; NCK2, Non-catalytic region of tyrosine kinase adaptor protein 2, SULT1B1, Sulfotransferase family 1B member 1; FAT-1, Protein fat homolog; ZO-1, Zona occludens-1; Arp2, Actin-related protein 2; Arp3, Actin-related protein 3; VEGFR2, Vascular endothelial growth factor 2; POD1, Podocyte expressed 1; MAFB, Kreisler (Mouse) Maf-related leucine zipper homolog; CLIC5, Chloride intracellular channel 5; ACTN4, Actinin alpha 4; MPP5, Membrane protein, palmitoylated 5; NTNG1, Netrin G1].

body, major, secondary, and foot processes, commonly called the arborized morphology of podocyte [4]. The functional unit of the glomerular filtration barrier (GFB) is formed by interdigitating glomerular podocytes and capillary endothelial cells. In the foot processes of podocytes, the major protein is filamentous actin (F-Actin) while at cell-cell junction multi-cell shape proteins makes a complex called as the slit diaphragm (SD). These adapter proteins include P-CDH, Podocin, Nephrin, CD2-associated protein (CD2AP), Non-catalytic region of tyrosine kinase adaptor protein 1/2 (NCK1 and NCK2), and the atypical protein kinase C-Partitioning defective 3 homolog-Partitioning defective 6 homolog complex (aPKC-PAR3-PAR6), Protein fat homolog (FAT-1), Zona occludens-1 (ZO-1)/Tight junction protein-1 (TJP1), and actin-related proteins (Arp2 and Arp3) [5].

In vitro generations of functional cells are required for treating renal disease or for the drug targets of patient-specific cells in personalized medicine. Podocyte can be generated by direct reprogramming via generation of NPs or directed reprogramming via podocyte progenitors or terminally differentiated podocytes [6]. Efforts for potential generation of podocyte progenitors and mature glomerular podocytes in vitro have been discussed and summarized in **Figure 1**. In this review, the term "Podocyte Progenitor," reflects those NPs that expressed nephron lineage and specific podocyte markers. The podocyte in kidney organoid formation has not been emphasized here. The kidney organoid structure required multiple cell type generations through the use of the different scaffolds which organized in renal epithelial cells likely in the complex architecture of the kidney for which a separate in-depth discussion is required.

Podocyte differentiation and characterization tools

Putative glomerular podocyte differentiation procedures have been developed in the last decade. The culture conditions for lineage commitment or specialized cells types conversion direct signal transduction mechanism. The

Table 1. The summary of the protocols for the differentiation of stem cells into the nephron progenitor (NPs) and the podocyte progenitors

Original cells	W9.5 ESCs (P18) derived Pax2-GFP$^+$ mESCs	mESCs derived EBs	hiPSCs derived from human kidney mesangial cells	H6 cells (CD-1 mouse's Pax2$^+$ KSCs)	HK2 (Human kidney cell line-adult proximal tubule cells)
Number of cells	EBs from W9.5 ES cells (P18) (1×10^5/ml, 2×10^3/ml), EBs derived Pax2-GFP$^+$ ESCs (8×10^4/ml)	-	-	-	-
Differentiation culture conditions (small molecules/growth factors/transcription factors/micro RNA)	Stepwise: ME: DMEM, 1% methylcellulose, BMP4 (2 ng/ml) IM: BMP2, BMP4, BMP7 each with (0.5, 5, 50 ng/ml), Chordin (1.25 µg/ml), BMP2, BMP4, BMP7 (5 ng/ml), serum & serum free conditions	Stepwise: mESCs on mouse feeder layer then on 1% agar within 2 days to form EBs then for 6 days trypsinized EBs on 0.1% gelatin, DMEM, 15% FCS, L-glutamine (2 mM), β-Mercaptoethanol (5×10^{-5} M), NEAA (1:100), RA (10^{-6}, 10^{-7}, 10^{-8}), A A (1, 5, 10 ng/ml), 10th day, UB cells of gestation day 13 fetal kidney conditioned medium for further 10 days	DMEM-F12, 2.5% FBS, β-Mercaptoethanol (100 µM), 0.1% gelatin coated dishes. AA (10 ng/ml), RA (0.1 µM), BMP7 (15 ng/ml)	High glucose DMEM, 2 mM glucose, 20% FBS, No other molecules. Spontaneous differentiation	GFP-Lentivirus for 7 days + VPA (2 mM). Combinatorial screening of 15 different factors for NPs (Six1, Six2, Pax2, OSR1, CRYM, N-NYC, cMYC, HOXA11, EYA1, SNAI1, SNAI2, WT1, MEOX2, HMGA2, OCT4)
Endpoint duration of analysis	16th & 19th day	10th & 20th day	•SEM on 10th day •Expension of differentiated cells + 10 days without small molecules & growth factors	4 weeks	6 days
Detection methods/ characterization	•ICC (Pax2$^+$ in EBs) •qRT-PCR (*α-Globin, β-h1globin, Aqp1, Brachyury, GDNF, GFP, HPRT, CDH16, LacZ, Mineralocorticoid receptor, NPHS1, OCT4, Pax2, PODXL, Pod-1, Wnt4, Wnt11, WT1*) •FACS (Pax2 GFP$^+$, PI)	•ICC IM: (Brachyury, Pax2) Renal genes: (WT1, E-CDH, POD-1, DB, Pax2) •Flow cytometry (Brachyury, Pax2)	•SEM •ICC (Nephrin, Podocin, Pax2, WT1, SYNPO)	•ICC (Pax2, WT1, αSMA, CK8, GDNF, Musahi1, Nanog) •RT-PCR (*Pax2, WT1, GDNF, Sall1, PODXL, SYNPO, UMOD, Desmin, Megalin, AQP1, AQP2, CK8, Six2, Nanog*)	•ICC (10 pools were identified based on induction of CITED1. further screening identified pool 8 by qRT-PCR) •qRT-PCR (*Six1, Six2, OSR1, Pax2, HOXA11, EYA1, SNAI2, CITED1, E-CDH, MMP2, MMP9*) •RT-PCR (*Six2, CITED1, Sall1, FOXD1, GSC, FOXA2, PAX6, NANOG*) •ICC (Pax2, CITED1)
Functional analysis	-	-	•Cell contractility assay (AII, RFP-actin, RFP-talin) •Cell permeability assay {FIT-C labeled albumin (0.5 mg/ml)} •Re-aggregation assay (mouse embryonic kidneys, E 13.5-15.5)	•Alkaline phosphatase staining for proximal tubular cells •No specific functional assay for podocytes like cells •Flow cytometry for stem cells markers (Sca1$^+$, CD24$^+$), mesenchymal markers (CD29, CD44) hematopoietic markers (CD45, CD117)	•Recombination assay E12.5 mouse kidney + GFP$^+$ single cells, detected by •ICC (WT1$^+$, Six2$^+$, Calbindin$^-$)
Uni/multi progenitors	Multipotent progenitor from IM to renal lineage cells	Multipotent progenitor from IM to renal lineage cells	Unipotent, podocyte progenitors	Multipotent progenitor to podocytes, mesangial cells, & proximal tubular cells.	Nephron progenitors
References	[7]	[8]	[11]	[21]	[22]

Original cells	hESCs {HES3 (MIXL GFP/wt)}	hESCs-H9 cell line, hiPSCs-derived from CRL2097 fibroblast	hPSCs {iPSCs (Fibro-epi)}, ESC (H1) human fibroblast episomal derived	hiPSCs (SC101A-1) clone IV derived EBs	Sprague Dawly rat's BM-MSCs, AD-MSCs
Number of cells	12000-15000 cells/cm^2	-	~300-500 cells/colonies	-	-
Differentiation culture conditions (small molecules/growth factors/transcription factors)/micro RNA)	Stepwise: PS: 2-3 days matrigel coated dishes, 1. BMP4 (30 ng/ml), AA (10 ng/ml), or 2. BMP4 (30 ng/ml), CHIR99021 (8 μM), serum free APEL media. IM: 4 days in FGF9 (200 ng/ml), heparin (1 μg/ml). further differentiation 4-11 (6 days) for 1. FGF9 (200 ng/ml), BMP7 (50 ng/ml), RA (0.1 μM), Heparin (1 μg/ml). 6 days For 2. FGF9 (200 ng/ml), Heparin (1 μg/ml). Cultured for further 6 days	Stepwise: Serum & feeder free system PS: 3 days {1 day, AA (100 ng/ml), Wnt3a (100 ng/ml), IM: 2 days, BMP4 (20 ng/ml), bFGF (10 ng/ml)} IM: 6-8 days, RA (10 μM), BMP7 (50 ng/ml), bFGF (10 ng/ml). 15 days NP: BMP7 (150 ng/ml), bFGF (50 ng/ml). For all above, the medium RPMI-1640 containing 2% B27, L-glutamine (2 mM), 1% PenStrep. For podocytes differentiation NPs cultured on fibronectin-coated dishes in VRAD medium (DMEM-F12, RA (100 μM), 10% FBS) for 7 days	Stepwise: IM: 2 days on growth factor reduced matrigel-coated plates, DMEM-F12, BSA (17.5 mg/ml), hInsulin (17.5 μg/ml), h holo-transferin (275 μg/ml), Monothioglycerol (450 μM), L-glutamine (2.25 mM), NEAA (2.25 mM), Penicillin (100 units/ml), Streptomycin (100 μg/ml), bFGF (50 ng/ml), hBMP4 (30 ng/ml), For further 2 days in ATRA (1 μM), hAA (10 ng/ml), BMP2 (100 ng/ml) + same medium	Stepwise: ME: 0-3 days, IM: 3-6 days, MM: 6-12 days, NPs: DMEM-F12, 5% FBS, NEAA (0.1 mM), β-Mercaptoethanol (0.1 mM), ATRA (0.1 μM), CCG1423 (1 μM), LY294002 (5 μM) till day 6; AA (10 ng/ml) for day 2-4. For 6-19 days, BMP7 (50 ng/ml), FGF2 (10 ng/ml), GDNF (15 ng/ml)	Fibronectin coated dishes, DMEM low glucose, 15% FBS, FGF2 (50 ng/ml), TGFβ2 (4 ng/ml), LIF (20 ng/ml)
Endpoint duration of analysis	2-18 days	15 days NPs. Later Podocyte formation	1-6 days	0, 6, 12, & 19 days	7 days
Detection methods/ characterization	• FACS PS: {Post 3 days, (2 × 10^6 Cells) MIXL1-GFP+} • ICC (Pax2, OSR1, LHX1, TBX6, SOX17, Six2, E-CDH, WT1, HOXD11, GATA3, JAG1) In this quantification proportion of induced cells (Pax2, LHX1, Sox17, Six2, WT1, CDH6, SYNPO) • qRT-PCR, day 3 PS: {*SOX17, Brachyury (T) MIXL1*} day 6 IM: (*Pax2, LHX1, FOXF1, TBX6*) • RT-PCR (*Pax2, LHX1, OSR1*) • RT-PCR for day 0-17 PS: (*MIXL1, LHX1)* IM: (*LHX1, Pax2, OSR1*) MM: (*OSR1, SIX2, WT1, GDNF, HOXD11*) UE: (*PAX2, CRET, HOXB7*) Ectoderm: (*PAX6*) • Pellet IMF (CALB1, AQP1, AQP2, SLC3A1, HuMt, HuNu)	• qRT-PCR PS: (*T, MIXL1, EOMES*) Endodermal: (*SOX17, FOXA2*) Ectodermal: (*PAX6, SOX1*) IM: (*OSR1*) NPs: (SIX2, *WT1, GDNF, HOXDA11)* Metanephric stroma/UB: (*FOXD1, HOXB7. Bone, RUNX2, COL1A1)* Vascular endothelium*: (PCAM1, TIE2)* Smooth Muscle: (*MYH11, CALPONIN)* Liver: *(ALB, AAT)* Neuron: (*TUJ1, MAP2)* Tubular: (*SLC12A3, CD13, AQP1)* Podocyte: (*SYNPO, Nephrin)* • RT-PCR IM: (*OSR1, PAX2, SALL1, EYA1, WT1)* NPs: *SIX2, CITED1, OSR1, PAX2, SALL1, EYA1)* • ICC (T, TRA1-81, OCT4, OSR1, PAX2, SALL1, SIX2, WT1, E-CDH, ZO1, KRT18, F-ACTIN, CD13, AQP1, MUCIN1, SYNPO, PODXL)	• qRT-PCR IM: {*T, OSR1, LHX1(LIM1), Pax2, Pax8, GATA3, OCT4, Nanog, SOX3}* IM ureteric progenitor-like cells: *(Six2, GDNF, WT1, Sall1, Cited1)* UB: *(HOXB7, RET, GFRA1)* • ICC IM: (HuNu, CK8, Six2) IM ureteric progenitor-like cells: {(Six2, WT1, LHX1 (LIM1)}	• ICC (OSR1, WT1, Pax8, Pax2, Six2, Sall1, CD133, CD24, NCAM, Claudin1, AQP1, GGT1, SSEA4, TRA1, Nanog, T, AFP, Pax6, Nkx2.5) • FACS (TRA-1-81) • qRT-PCR human (*POU5F1, Nanog, DNMT3B, GABRB3, GDF3, SOX2, TDGF1, RAF1, ELF1, T, LHX1, OSR1, SIX2, PAX8, NANOG, SALL1, WT1, PAX2*)	• ICC (Wnt4, WT1, Pax2, Vim, Oct4, Sox2, E-CDH, ZO-1) • qRT-PCR (*Pax2, Wnt4, WT1, E-CDH, ZO-1*)
Functional analysis	• 3D culture (10 × 10^5 cells), collagen IV coated (10 μg/cm^2) filter membrane (0.4 μm) • Re-aggregation assay, embryonic kidneys (12.5-13.5 dpc) collagen IV coated (10 μg/cm^2) filter membrane	• Alkaline phosphatase staining for tubular cells • In vitro tubulogenesis assay • No functional assay for podocytes	• 3D organ co-culture assay • qRTPCR for above {(*T, OSR1, HOXB7, LHX1 (LIM1), Pax2, GFRA1*)} • ICC (CK8, HuNu, Six2, ZO1)	• Cisplatin-induced AKI model • HC (H&E, PAS) • IHC (HNA, hMitochondria, AQP1, WGA lectin, PNA lectin, AQP3, Ki67)	-
Uni/multi progenitors	Multipotent progenitors, ureteric & metanephric progenitors	Multipotent progenitor to podocytes, & tubular cells	Ureteric bud committed renal progenitor-like cells	Multipotent progenitor	Nephron progenitors
References	[9]	[10]	[12]	[14]	[19]

capacity of stem cells potentiates this mechanism. Currently, transcription factors (TFs), small molecules, and growth factors are the three well-known factors, which boost the process for progenitor population, specialized cells formation, and kidney organoids development. Studies have been conducted to potentiate the productivity and efficiency of differentiated podocyte, for example, by utilizing micro ribonucleic acids (miRNAs) technology. Starting origin, the number of cells, duration of differentiation, detection methods, the method of characterization, and functional analysis, although varied in in vitro differentiation protocols. However, many of them showed the generation of functional podocyte according to the in vivo counterpart. In vitro, up-to-date protocols for nephron and podocyte progenitors and differentiated glomerular podocyte have been summarized in **Tables 1**, **2**.

NPs and podocytes are derived from different sources like embryonic stem cells (ESCs) [7-10], induced pluripotent stem cells (iPSCs) [11-17], hematopoietic stem cells (HSCs) [18], adipose-derived mesenchymal stem cells (AD-MSCs) [19, 20], kidney-derived stem cells (KSCs) [21], and kidney cell line [22]. Almost all protocols used immunofluorescence analysis by immunocytochemistry (ICC), flow cytometry; polymerase chain reaction (PCR), and quantitative real time polymerase chain reaction (qRT-PCR) for the characterization of generated cells. Functional analysis of these protocols includes cell contractility via Angiotensin II (AII), cell permeability assay or albumin uptake assay [11, 15, 16] for the perinuclear accumulation of albumin [15], and scratch assay for the migration of cells [18]. The re-aggregation assay or recombination assay or chimeric organoid cultures utilized embryonic Kidney's cells of 12.5-13.5 days post coitus (dpc) [9, 11, 20, 22]. Three dimensional (3D) organ co-cultures methods were utilized to grow cells similar to in vivo condition to observe organ niche integration, interaction, and the generation of the response of the cells [9, 12]. In vitro generated cell transplantation was also carried out in the kidney capsule [13, 20]. Urinary protein excretion levels [20], cytoskeletal examination by F-actin rearrangement [15], negative expression of the ectodermal and endodermal genes, which does not induce *Pax6, NES, SOX17, ALB, ACTA2*, and *α-SMA, FOXD1* respectively in reprogrammed cells, further validate the results [12]. However, some of the studies did not contribute to the functional aspects of the kidney in newly developed cells [7, 8, 19]. Sequencing data of single cell analysis characterized the progenitor and mature podocyte by the expression of *LHX1, EMX2, JAG1,* and *NPHS1, NPHS2, CLIC5, PODXL, SYNPO, VEGF, MPP5, TJP1, NTNG1, MAFB* respectively [23].

Direct programming by transcription factors

Regulations of cellular processes are governed under coordination between target genes and proteins. Specific regulatory proteins are TFs that bind to deoxyribonucleic acid (DNA) through their DNA-binding domains (DBDs). The sequences on the DNA are termed transcription factor binding sites (TFBS) [24, 25]. Remodeling of cells is associated with transcription levels driven by TFs. The direct approach for reprogramming is the forced or exogenous expression of key TFs to change the identity of cells into the desired cells. Stable transcription of glomerular podocyte specific genes can maintain the gene expression and capture the phenotype and function of podocyte. Complete TFs for cognate DNA elements and the correct combination of a few specific TFs for converting stem cells or fibroblast into podocyte are still unknown. However, some strategies have been utilized and new combinations are continuously evolving [6, 22, 26]. Two approaches for transporting TFs were frequently practiced that is non-integrating (chemicals, physical) and integrating (retro-lentiviral expression system) [22].

Podocytopathies are caused by genetic mutations in TFs, signaling mediators, and SD proteins. These mutations and mesenchymal to epithelial transition (MET) during development can provide clues for targeted protein expression for in vitro differentiation of podocyte. For characterization, WT1 and Nephrin are specific podocyte markers as they do not express in other nephron's cell types. Cell adhesion proteins cadherins (CDH) are focal for specification and characterization of cells types. Mature podocytes do not have epithelial cadherin (E-CDH) but express P-CDH, while N-CDH expressed upon TGF-β1 treatment [1]. Although no reports for the kidney, in situ direct reprogramming of functional regenerative cells by delivering specific TFs have been reported in the mice

Table 2. The summary of the protocols for the differentiation of stem cells into the glomerular podocytes

Original cells	hiPSCs	hiPSCs (Episomal iPS cell lines)	PGP1 hiPSCs cell line	$CD34^+$ hHSCs	hAD-MSCs
Number of cells	NPHS1-GFP^+ iPSCs (201B7)	30,000/50,000 cells/cm^2	4×10^4 cells/cm^2	1×10^3 cells/cm^2	5×10^5 cells/well of 6 well plate
Differentiation culture conditions (small molecules/growth factors/ transcription factors/micro RNA)	iNPs aggregates, 0.8 μM polycarbonate filter, DMEM, 10% FCS, mouse embryonic spinal cord (E12.5), clone 3 on feeder free condition	Stepwise: ME, IM, NP, Podocytes. ME: Growth factor reduced matrigel coated dishes, For 3 days DMEM-F12, 2.5% FBS, GlutaMax (1:1), neurobasal media + N2B27 + CP21R7 (1 μM), BMP4 (25 ng/ml). IM: first medium replaced by STEMdiff APEL medium {RA (100 nM), BMP7 (50 ng/ml), FGF9 (200 ng/ml) for 2 days (total 5 days)}. NPs: 6^{th} day for 7 days passaged by Accutase plated on type1 collagen-coated plates at a density of 20,000/40,000 cell/cm^2 in VRAD medium {DMEM-F12 plus GlutaMax, 10% FBS, RA (80-100 μM), Vitamin D3 (100 nM)}	Stepwise: ME, IM, mature podocytes. On ECM (laminin 511-E8-coated plates). For 2 days in ME medium *i.e.* DMEM-F12 + GlutaMax, AA (100 ng/ml), CHIR99021 (3 μM), Y27632 (10 μM), 1X B27 serum free supplement. IM medium for 14 days DMEM-F12 + GlutaMax, BMP7 (100 ng/ml), CHIR99021 (3 μM), 1X B27 serum free supplement. Split cells 1:4 on ECM for 4-5 days in podocyte medium . DMEM-F12 + GlutaMax, BMP7 (100 ng/ml), CHIR99021 (3 μM), BMP7 (100 ng/ml), AA (100 ng/ml), VEGF (50 ng/ml), RA (0.1 μM), 1X B27 serum free supplement	Stepwise: For 5 days AA (10 ng/ml), RA (2.5-10 ng/ml, optimum 7.5 ng/ml), BMP7 (2.5-10 ng/ml, optimum 5 ng/ml) resulted $OSR1^+$ cells. These cells for 9 days AA (10 ng/ml), RA (7.5 ng/ml), BMP7 (5 ng/ml), EGF (20 ng/ml), bFGF (20 ng/ml)	Stepwise: IM: for 3 days DMEM-F12, 2% FBS, AA (10 ng/ml), RA (10 μM). Three types of culture conditions + same basal medium 1. AA (10 ng/ml), RA (0.1 μM), BMP7 (20 ng/ml) 2. AA (10 ng/ml), RA (0.1 μM), GDNF (20 ng/ml) 3. AA (10 ng/ml), RA (0.1 μM), Wnt4 (50 ng/ml) • Transfection by lipofectamine2000 • mi-RNA selection, miRNA-498 by TargetScan & Pictar algorithm
Endpoint duration of analysis	Day 9	ME day 2, IM day 4, NP day 6, mature podocytes day 13	21 days	14 days	Day 9
Detection methods/characterization	• ICC (WT1, E-CDH, CDH-6) (NPHS1, WT1, PODXL) • IHC (H&E) Day 9 (Nephrin, GFP, WT1, Type IV collagen, E-CDH, CDH6, PODXL, CD31, human nuclear antibody) • Flow cytometry (Nephrin, PODXL) • qRT-PCR (*WT1, NPHS1, NPHS2, SYNPO, PODXL*) • Microarray • SEM • TEM	• ICC ME: (Oct4, T) IM: (Pax2, OSR1, LHX1) NP: (Pax2, Six2, WT1) • PCR ME: (*T, Nanog*) IM: (*Pax2, OSR1, LHX1*) NPs: (*WT1, SYNPO, NPHS1, ACTN4, CD2AP, VEGF-A*) • qRT-PCR (*Six2, ACTN4, HPRT*) • SEM, day 6	• Flow cytometry (Oct4, WT1, Nephrin) • ICC (Nephrin, WT1, Pax2, Podocin, Oct4, OSR1, EdU, PKCλ/I, Collagen type IV, FcRn receptor for albumin & IgG transport) • qRT-PCR (*POU5F1, Pax2, WT1, NPHS1, NPHS2*) • Western blot (PKCλ/I) • SEM	• ICC (Podocin, SYNPO, GLEPP1), post 3 days (Pax2, WT1) post 9 days (Pax2, NPHS1, SULT1B1, NPHS2, SYNPO) • Leishman's staining • Western blot (Podocin, SYNPO) • Flow cytometry (CD45, CD34) • SEM	• ICC (OSR1, WT1, Pax2, Podocin, Nephrin, SYNPO, Laminin, HNA) • Flow cytometry (OSR1) • qRT-PCR (*miR-498*) • Western blot (Podocin, Nephrin, SYNPO, WT1)
Functional analysis	• Transplantation of cells using solid agarose rods under NOD/SCID/JAK3 null mice kidney capsule, post 20 days characterized by • HC (H&E) • IHC (WT1, CD31)	• Cell proliferation assay (differentiated vs. undifferentiated) • Cytoskeleton rearrangement evaluation (peripheral localization of F-actin) • Albumin uptake assay • Chimeric organoid cultures (E12.5 CD1 mouse) • ICC (E-CDH, HNA, WT1)	• ICC EdU-incorporation assay • Albumin uptake assay (confocal imaging & quantification of albumin positive cells) • SEM	• Tyrosine kinase assay • Scratch assay (1×10^6 cells on 0.8% agarose molds)	• Re-aggregation assay (E12.5) by HC, ICC (WT1, Laminin, Nephrin, HNA, Podocin) • Estimation of urinary protein excretion (Adriamycin induced-model in Balb/c mice)
References	[13]	[15]	[16]	[18]	[20]

models of cardiomyocytes in myocardial infarction, endocrine beta cells, neurons, and hepatocytes [6]. In situ direct programming methods, their efficiencies, and safety methods are required to optimize for the renal therapy in humans.

A major technology to examine the genome-wide binding of TFs is chromatin immunoprecipitation (ChIP) followed by deep sequencing (ChIP-seq) but only limited TFs were identified by ChIP-Seq for podocyte differentiation. Dynamic motif occupancy analysis (DynaMo) is an algorithm to accurately predict the spatiotemporal binding pattern of TFs responsible for the dynamic process. This program has been utilized for human neural differentiation [24] and other studies can also be carried out using this tool, but it exhibits no nephron related study. JASPAR (http://jaspar.genereg.net) is an open-access database of TF-binding profiles, which are stored as position frequency matrices (PFMs). A PFM summarizes experimentally determined DNA sequences bound by individual TFs [25]. Direct programming can utilize a cocktail of TFs which can yield high efficiency of a homogeneous population. Novel targeted TFs binding sites in the genome can be identified through a computational tool, protein interaction quantitation (PIQ) (http://piq.csail.mit.edu) at corresponding motifs from deoxyribonuclease I (DNase I) hypersensitive sites sequencing (DNase-Seq) experiments with accuracy comparable to ChIP-Seq. This technique utilizes DNaseI hypersensitivity profiles. It also models the magnitude and shapes of genome-wide DNase profiles to facilitate the identification of TF-binding sites. It consists of three steps: candidate site identification, the background model computation, and TF binding estimation [26]. Mogrify and CellNet, which can select candidate factors for cell fate decisions, are other computational programs and prediction methods [6].

Directed programming by small molecules and growth factors

Small molecules and growth factors combination create a synthetic niche for induction, to maintain differentiation potential for the expansion and propagation of newly developed cells. The directed or instructive signaling cues towards podocytes generation utilized chemically defined culture conditions, which are comprised of a basal medium with fetal bovine serum (FBS), rich in small molecules and growth factors. Small molecules like CHIR99021 [9, 16], activin A (AA) [8-12, 14, 16, 18], all-trans retinoic acid (ATRA) [8, 11, 12, 14-18, 20], Valproic acid (VPA) [22], CP21R7 [15], Y27632 [16], CCG1423, LY294002 [14], Chordin [7], and growth factors such as bone morphogenic protein (BMP) family (BMP2) [7, 12], BMP4 [7, 9, 10, 12, 15], BMP7 [7-11, 14-18, 20], Fibroblast growth factor (FGF) family, FGF2 [10, 12, 14, 18, 19], FGF9 [9, 15], Wingless/Integrated (Wnt) family Wnt4 [20], Wnt3a [10], Glial cell derived neurotrophic factor (GDNF) [14, 16, 20], Vascular endothelial growth factor (VEGF) [16], Epidermal growth factor (EGF) [18], Leukemia inhibitory factor (LIF), Transforming growth factor, beta 2 (TGFβ2) [19], have been utilized and function as the first messenger to produce signals to generate renal progenitor and glomerular podocyte. Small molecules serve as an alternative to TFs. Small molecules and growth factors provide non-integrative effects to initiate the renal development program for cell fate conversion. Chordin is a BMP antagonist whose complete function regarding podocyte differentiation is not known. RA and AA induce intermediate mesoderm (IM) and express OSR1, Pax2, and WT1. Sall 1 is expressed in metanephric mesenchyme (MM) and represent as NPs population for podocyte generation [10, 12, 14, 21, 22]. The major cellular functions of small molecules have been summarized in **Table 3**.

Growth factors control cell growth through cell proliferation, differentiation, survival, and migration. It contributes to renal metabolism and development of the kidney. It triggers the differentiation and proliferation of cells by activating specific receptors. A number of signal transduction receptors, including nuclear receptors, receptor tyrosine kinases (RTKs), and G-protein coupled receptors (GPCRs) play a crucial role to initiate this process. The outside-in signal mediates cell-matrix adhesion via Integrins [2] and regulates the differentiation of podocytes [27]. The differentiation studies showed that combinatorial programming was employed to produce nephron progenitor cells in two to three steps or induced a sequential programming as governed in the physiological program in kidney development that is starting from primitive streak (PS) formation followed to mesoderm (ME), IM, NPs like cells, and then

Table 3. Small molecules and their specific roles in the podocyte differentiation

Small molecules	Cellular functions	References
CHIR99021	• [1]GSK3β1 inhibitor • Wnt agonist	[9, 16]
Activin A (AA)	• Cellular homeostasis • Inducer of differentiation • Activator of cell differentiation and inhibitor of cell growth and proliferation	[8-12, 14, 16-18, 20]
All-trans retinoic acid (ATRA)	• Cellular homeostasis • Inducer of differentiation • Bind to [2]CRABP2 in the nucleus • Activate transcription of RA primary response genes	[8, 10-12, 14-18, 20]
Chordin	• [3]BMP antagonist • Development of the vertebrate gastrula	[7]
Valproic acid (VPA)	• [4]HDAC inhibitor	[22]
CP21R7	• [1]GSK3β1 inhibitor • Activate canonical [5]Wnt signaling.	[15]
Y27632	• [6]RHO/ROCK pathway inhibitor	[16, 17]
CCG1423	• A potent and specific inhibitor of Rho pathway signaling	[14]
LY294002	• Inhibitor of [7]PI3Ks	[14]

[1]Glycogen synthase kinase 3 beta1, [2]Cellular retinoic acid binding protein 2, [3]Bone morphogenic protein, [4]Histone deacetylase, [5]Wingless/Integrated, [6]Rho-associated coiled-coil forming protein serine/threonine kinase, [7]Phosphoinositide 3-kinases.

functional glomerular podocytes [6]. Defined culture conditions may retain the inductive signals and facilitate orchestration of events of in vivo nephrogenesis. This represents the graded cues activate Nodal/Activin and Wnt signalling, which guides the differentiation process of original cells. 3D aggregates, *i.e.* embroid bodies (EBS) [7, 14], which first expressed NPs population that were further turned to account either for renal tubules [28-34] or glomerular podocyte formation or both tubules and podocytes formation [10, 21]. Ciampi et al., 2016 showed that NPs at day six whiles at day thirteen mature podocytes were generated in the STEMDIFFApel medium containing RA (100 nM), BMP7 (50 ng/ml), and FGF9 (200 ng/ml). It showed the highest expression levels of Six2 on day six of the differentiation upon testing of five different conditions comprised of RA, BMP7, FGF9, STEMDIFF Apel medium vs. DMEM-F12 [15]. Patient's specific iPSCs derived progenitors can be utilized for clinical trials as it does not have ethical concerns like ESCs. Sharmin et al., 2015 induced iPSCs cell to podocyte and showed the overlap of human glomeruli and mouse podocyte marker expression [13]. Imberti et al., 2015 showed that up-regulated WT1 gene expression levels were also increased that can be utilized in the podocyte injury model to observe its integration into glomeruli. However, they operated NPs for renal tubule formation in the acute kidney injury model [14]. Podocyte progenitors could be very effective in ameliorating podocytopathies and can differentiate into mature functional podocytes in the niche of nephron and can replace non-functional podocytes. Similarly, functional podocytes are also considered to be essential for the urgent and the direct utilization of these cells in the cellular therapy of CKD. Matured podocytes are imperative to microfluidics/organ-on-a-chip technology for podocytes *i.e.* facilitates drug discovery and illuminates disease mechanism. Human clinical trials are required to observe ex vivo and in vivo potential to these cells [16, 17].

Signaling pathways of podocyte differentiation

BMP signaling is established during development. The BMP family members; BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, and BMP-7/Osteogenic protein1 are present in the kidney. Among all the BMP family members, BMP-7 is most abundant in human fetal and adult kidney [35]. BMP4 promote PS genes like *Brachyury (T), Mixli, Tbx6, Flk1,* and IM genes [7]. In the postnatal stage in mice, BMP4 expression was observed, but Bmp2 and BMP7 were decreased [36].

Wnt/β catenin signaling is crucial for nephron development. Wnt can activate three intracellular pathways: First, the canonical pathway; T-Cell factor (Tcf)/β-catenin, second the non-canonical (planar cell polarity), and third the Wnt/Ca^{2+} pathway. However, in adult kidney Wnt signaling turns to be silenced. The canoni-

cal pathway regulates β-catenin through glycogen synthase kinase-3β (Gsk/3β)/Wnt signaling. β-catenin with P-CDH maintains podocyte integrity by stabilizing cell adherens junctions [37]. Phosphorylated Nephrin is critical for podocyte function *i.e.* maintains its morphology [38]. Phosphoinositide 3-kinase (PI3K)/Akt play role in cell survival and regulation of integrity of actin stress fibers. The SD proteins, including Nephrin, Podocin, and CD2AP complex facilitate the maintenance of Akt phosphorylation by interacting PI3K [37, 38]. Insulin-like growth factor 1 (IGF-1), although did not utilize in any of the in vitro protocol discussed here, it has a protective effect in fetal podocytes through IGF-1 receptor (IGF-R) stimulation form insulin receptor substrate (IRS)-1-p85 complex, an increase in PI3K activity, protein kinase B (PKB/AKT), and reduced apoptotic protein Bad. It confers survival and maintenance of podocytes in vitro. Both Nephrin and CD2AP interact with the p85 regulatory subunit of PI3K and it stimulate AKT signaling with Podocin [39]. Hence, IGF-1 could be a contributing factor for podocyte differentiated cells for optimal survival in vitro.

The cyclic adenosine monophosphate (cAMP) pathway is involved in podocyte differentiation. RA induction stimulate Kruppel like factor 15 (KLF15) expression and binds to the promoter regions of Nephrin and Podocin, two critical differentiation markers. With the activation of protein kinase A (PKA) and cAMP-response element binding protein (CREB), RA attenuates podocyte dedifferentiation. GDNF, which is implicated in podocyte differentiation, is also CREB target and highly regulated by RA [40].

All kidney cells required the fibroblast growth factor receptor (FGFR) signaling, mainly for growth and patterning. Mature podocyte expresses several FGF molecules, including, FGF1, FGF2, FGF7, and FGF10 but FGF2 has mitogenic effects on podocyte by autocrine signaling and paracrine signaling through mesangial and endothelial cells. During podocyte differentiation, FGF2 proteins remain highly expressed in functional podocyte. It maintains cells in the induced state in absence of inductive signals [2]. FGF2 and BMP7 inhibit tubulogenesis and both promote stromal progenitor cell population during the differentiation process. FGF effect is enhanced by BMP7, and it prevents apoptosis and also up-regulate expression of WT1 in MM culture [41]. FGF signaling is critical for podocyte foot processes formation in differentiation-induced cytoskeletal reorganization via F-actin. In this process expression of slug and vimentin (VIM) evoke epithelial to mesenchymal (EMT) changes, which are necessary for terminal differentiation [42].

Adult kidneys do not express Notch signals. Notch pathway components are expressed and up-regulated in renal and podocyte progenitors throughout nephrogenesis and in glomerular diseases. Notch establishes the proximo-distal axis of a nephron. Notch stimulates S-phase entry and cell division in renal progenitors whiles its downregulation facilitate differentiation of podocyte. It may start abnormal mitosis (mitotic catastrophe) in podocyte. There are four single-pass transmembrane Notch receptors (Notch 1-4), and five ligands, Delta-like (Dll 1, 3, 4), Jagged 1 (Jag 1, 2) members of this family [43, 44]. Ligand binding results in Notch intracellular domain (NICD) activation by cleavage through c-Secretases and NICD nuclear translocation. NICD binds with recombination signal-binding protein-J (RBP-J) and activates downstream transcriptional target genes the Hairy enhancer of split (Hes) factors and its related repressor proteins (Hey). Hes control tissue-specific differentiation genes [43]. Podocyte progenitors express Notch 1, Notch 2, and downstream transcriptional targets Hes1, and Hey1 in the S-shaped body stage of glomerulogenesis. Gradually during terminal podocyte differentiation, which includes tertiary foot process assembly and SD formation, is in agreement with down-regulation of Notch pathway components. The deletion of Notch processing gene PSEN that encodes presenilin in murine kidneys, results in loss of podocytes and proximal tubules [45]. During terminal podocyte differentiation, Notch signals also regulate autophagy for podocyte differentiation as Notch 1 and autophagy increased simultaneously in this processes [44].

Podocytes have autocrine VEGF regulation. Isomeric VEGF-A and VEGF-C play the autocrine role in podocyte survival. Its inhibition provoke proteinuria [46]. Podocytes have no proliferation capacity in its matured form [47]. VEGF-A signaling regulates SD proteins by inducing a dose-response Podocin up-regulation and increase its interaction with CD2AP. The data indicate that podocytes in culture have a functional autocrine VEGF-A system that is regulat-

ed by differentiation and ligand availability. VEGF-A in podocytes promote survival through VEGFR2, induce Podocin up-regulation, and increases Podocin/CD2AP interaction [48]. Additional in vitro and in vivo studies are required for defining the role of VEGF during differentiation towards podocytes.

Role of podocyte cytoskeleton

The three parts of podocytes are the apical membrane, foot process, and basal membrane. These are maintained by the cytoskeletal organization, which includes microtubules, Vimentin rich intermediate filaments, and Actin proteins. All of these components maintain cell shape, rigidity, and cell motility. The positioning of membrane organelles and signals is transmitted through microtubules via protein vesicular transport along their tracks. Precise Actin cytoskeleton organization and regulation between cell-cell contacts are conferred normal structure and foot process movement of podocytes [5, 49]. The cortical Actin protein filaments are associated with many proteins as mentioned previously. Loss and gain podocyte's function is attributed to modeling actin as it supports SD proteins [46]. Therefore, many studies showed phosphorylated Synaptopodin (SYNPO) as a characterization tool, which acts as a stabilizer of Actin cytoskeleton [9-11, 15, 18, 20, 21]. Actin network in stress fibers of podocytes is controlled by Rass homolog family member A (Rho-A) and calcium pathways [50]. Rho A protein maintain an optimal degree of podocyte motility and its reduction is associated with hypermotility. Recent advances in imaging by multiphoton and light sheet imaging showed that reduction in podocyte's motility causes proteinuria in mice. It is produced when Rac1 and Cdc42, TRPC6 become inactive, and blockade of αvβ3 Integrin. While in disease condition hyperactivity of podocyte motility is associated with up-regulation of TRPC5, CatL in response to CD2AP deficiency. Phosphorylated SYNPO link to the Actin cytoskeleton and it binds to CD2AP and α Actinin-4, regulate Rho-A protein [5]. Up to date, there are no in vitro optimal motility functional assays available for the podocyte, which should have motility profiles as standard.

Role of micro RNA

miRNAs are single-stranded, non-coding RNAs molecules that negatively regulate or destabilized mRNAs via binding to its 3'-untranslated region. The roles of mi-RNAs have already been described for mechanisms like autophagy [51], apoptosis, proliferation, and differentiation [52]. Several mi-RNAs have been identified in physiologic and pathologic conditions of kidney [53]. miR-26a-5p levels were found to be lowered in lupus nephritis or IgA nephropathy [54, 55]. FSGS can be induced by an up-regulation of miR-193a-5p, which downregulates WT1 [56]. However, limited studies have been conducted on the role of miRNAs in podocytes as a regulatory molecule in its differentiation. The miR-200 family has five members organized as two clusters, miRs-200a/b/429, and miRs-200c/141 abundantly found in the kidney and expressed in pronephros [57]. Initially, it was found to be involved in renal fibrosis and diabetic nephropathy [58]. Later miR-200 family found to promote podocyte differentiation. miR-200a, miR-200b, and miR-429 significantly upregulated during podocyte differentiation with optimal expression of miR-200a. The miR-200 family directly inhibit radical S-adenosyl methionine domain-containing protein2 (RSAD2) also known as Viperin or Cig5, an anti-viral protein induced by interferon. The structural integrity of podocytes played a central role in maintaining the normal function of GFB [57]. miR-30a-5p and miR-193a-5p, maintain the phenotypic marker expression of podocytes [59, 60]. miRNA act through several podocyte adapters and effector proteins, and linked to the Actin cytoskeleton, for example; miR-155-5p enhanced Nephrin acetylation, which attenuates renal damage in hyperglycemia-induced nephropathy [61]. miRNA-498 inhibition improves human hAD-MSCs differentiated into podocytes. These cells were used in two steps to induce podocyte, first in IM by the application of AA and high concentration of RA, and then by the low concentration of RA and BMP7. The functional characterization was analyzed by embryonic explant culture and Adriamycin-induced injury model that showed integration capacity and reduction in proteinuria respectively. This method has been summarized in **Table 2** [20].

Excellence in in vitro differentiated cells

Quality measures are attributed to characterization techniques, the identification, and the use of specific gene expression compared to adult and developmental stages vs. in vitro remodeled cells. The major obstacles for cellu-

lar therapy in directed differentiation are a risk for teratogens and incomplete phenotypic resemblance of newly generated cells and its function. Incomplete characterization shows residual features of the originating population or has a non-homogenous population. The remainders of in vitro generated cells were not discussed in many published articles. After differentiation, a small number of pluripotent stem cells may produce heterogeneous cells. Therefore, the choice of original cell type is critical for in vitro differentiation. Complete native cell's transcriptomic and epigenetic studies may further enhance the information to resolve this problem. Multiplex gene and protein data may provide efficient quality control for cell fate conversion towards podocyte.

The in vitro programming methods require attention towards nanoarchitecture of glomerular basement membrane (GBM) in 3D culture. Podocyte in healthy glomerular tissue exists with physiological substrate stiffness, *i.e.* native GBM of the capillary that provides mechanical support [62]. Freeze-fracture of GBM in the scanning electron microscope (SEM) shows its porous nanotopgraphy, which supports filtration mechanism [4]. This phenomenon should include in in vitro differentiation protocols of podocytes as it can effect changes in podocyte phenotype, maturation, and filtration function. Podocyte iconic gene WT1 expression was observed as a podocyte mechano responsive gene in presence of transglutaminase microbial gelatin (gelatin-mTG) in the hydrogel culturing system [62]. Various surfaces can be employed for in vitro generation of podocyte considering the geometrical nanoporous surface, specifically for podocytes mimicking the in vivo conditions. Zennaro et al., 2016 showed that porous surfaces allow cytoskeletal remodeling and formation of focal contacts of podocyte. Actin reorganization and microtubule assembly via microtubule-associated protein 2 (MAP2) and Tau stabilized podocyte structure [4]. Besides soluble cues from growth factor and cytokine-mechano-transduction mechanism can cause the change in gene expression, which ultimately direct differentiation. Allylamine (AA) and Octadiene (OD) (low AA) composed homo-and co-polymeric plasma coatings surfaces directed differentiation towards glomerular podocytes and proximal tubules by the appearance of WT1, Nephrin, and Megalin respectively [27].

Future endeavor

Studies are required in efforts to generate efficient podocyte progenitor or mature podocyte. Functional assays are of a great magnitude to observe the programmed cells. The more likely models should be designed in combination to generate podocyte with the capillary network or endothelial cells to closely observe the filtration's mechanism. Secretion of growth factors like Notch 2 and VEGF-A, which affect capillary development as well as the differentiation of endothelial cells can be exercised as a characterization tool [63]. Purposive podocytes differentiation can be carried out such as α3ß1 Integrin expression as it is essential for the regulation of foot process assembly [12]. Insulin signaling is crucial for podocytes function. It is involved in the activation of AKT by Nephrin-dependent pro-survival cascade and the regulation of Actin cytoskeleton [2]. Advanced podocyte motility models in vitro similar to physiological conditions are lacking and required more attention not only to drive highly efficient podocytes but to observe novel pathology. Electrophysiological properties of podocyte should be monitored to control the contractile state of the foot process. Developing podocyte expressed Scribble, a protein that translocates from the lateral aspects of immature podocytes to the basal cell membrane and foot processes of mature podocytes [64]. This phenomenon can be observed in vitro in the progenitor or differentiated podocytes for validation experiments.

Concluding remarks

Highly efficient and homogeneous podocyte's progenitor and mature podocyte generation is the first and crucial step for either in vivo direct cell replacement therapy or ex vivo functional kidney development as the final treatment strategy for CKD patients instead of RRT. The protocols for procuring podocyte are being improved by the usability of the signal targeted TFs, small molecules, growth factors, and inhibition strategies through miRNAs. Identification of a specific combination of TFs and miRNAs for in vitro differentiation of podocyte is the current need to explore a dynamic process for programming. Monitoring podocyte cytoskeletal re-organization facilitates phenotypic change and cell fate conversion. The combination of

novel in vitro nanoarchitecture of GBM in 3D culture with podocyte motility analyzing methods would provide a new insight into the functional improvement of programmed differentiated podocyte from various cells sources.

All acronyms are mentioned in Supplementary Table 1.

Acknowledgements

This review article is supported by the grant from Sindh Institute of Urology and Transplantation (SIUT), Karachi-Pakistan.

Disclosure of conflict of interest

None.

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Supplementary Table 1. The List of Acronyms and the Nomenclature of Small Molecules

Acronyms	
AII	Angiotensin II
AA	Activin A
AA	Allylamine
AAT	Aspartate transaminase
ACTA2	Actin, alpha 2, smooth muscle, aorta
ACTN4	Actinin alpha 4
AD-MSC	Adipose mesenchymal stem cells
AFP	Alpha-fetoprotein
AKT	Akt/Protein kinase B
ALB	Albumin
cAMP	Cyclic adenosine monophosphate
APEL MEDIA	Animal product-free medium
AQP1	Aquaporin 1
AQP2	Aquaporin 2
AQP2	Aquaporin 3
Arp2	Actin-related protein 2
Arp3	Actin-related protein 3
Atf2	Activating transcription factor 2
ATRA	All trans retinoic acid
BM-MSC	Bone marrow mesenchymal stem cells
BMP2	Bone morphogenic protein 2
BMP3	Bone morphogenic protein 3
BMP4	Bone morphogenic protein 4
BMP5	Bone morphogenic protein 5
BMP6	Bone morphogenic protein 6
BMP7	Bone morphogenic protein 7
BSA	Bovine serum albumin
CALB1	Calbindin 1
CatL	Cathepsin L
CCG1423	N-[2-[(4-Chlorophenyl)amino]-1-methyl-2-oxoethoxy]-3,5-bis(trifluoromethyl)benzamide
CD2AP	CD2-associated protein
CDH	Cadherins
CNN	Calponin
CD13	Cluster of differentiation 13
CDH16	Cadherin 16/kidney specific protein (Ksp)
CD24	Cluster of differentiation 24
CD31	Cluster of differentiation 31
CD133	Cluster of differentiation 133
Cdc42	Cell division control protein 42 homolog
CG	Collapsing glomerulopathy
ChIP	Chromatin immunoprecipitation
ChIP-Seq	Chromatin immunoprecipitation-sequencing
CHIR99021	6-[[2-[[4-(2,4-Dichlorophenyl)-5-(5-methyl-1H-imidazol-2-yl)-2-pyrimidinyl]amino]ethyl]amino]-3-pyridinecarbonitrile
CITED1	Cbp/P300 Interacting transactivator with Glu/Asp rich carboxy-terminal domain 1
C-Jun	Transcription factor activator protein 1 (AP-1)/Jun proto-oncogene
CK8	Cytokeratin 8
CKD	Chronic kidney disease
CLIC5	Chloride intracellular channel 5
c-MYC	c-myc protein
COL1A1	Collagen, type I, alpha 1
CP21R7	3-(3-aminophenyl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione
CRABP2	Cellular retinoic acid binding protein 2
CREB	cAMP-response element binding protein
CRYM	Crystallin, mutated

DB	Dolichos biflorus
DBDs	DNA binding domains
Dll	Delta-like
DMEM	Dulbecco's Modified Eagle Medium
DMEM/F:12	Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12
DNA	Deoxyribonucleic acid
DNase I	Deoxyribonuclease I
DNase-Seq	DNase I hypersensitive sites sequencing
DNMT3b	DNA (cytosine-5-)-methyltransferase 3 beta
Dpc	Days post coitum
DynaMo	Dynamic motif occupancy analysis
EBSs	Embroid bodies
E-CDH	Epithelial cadherin [CDH1-cadherin 1, type 1, E-cadherin (epithelial)]
ECM	Extracellular matrix
EdU	5-Ethynyl-2'-deoxyuridine
EGF	Epidermal growth factor
ELF1	E74-like factor 1 (ets domain transcription factor)
EMX2	Empty spiracles homeobox 2
EMT	Epithelial to mesenchymal
EOMES	Eomesodermin
ESCs	Embryonic stem cells
Ets1	Transcription factor/avian erythroblastosis virus E26 (V-Ets) oncogene homolog-1
EYA1	Eyes absent (Drosophila) homolog 1
FACS	Fluorescence-activated cell sorting
FAT-1	Protein fat homolog/cadherin-related family member 8
F-Actin	Filamentous actin
FBS	Fetal bovine serum
FCS	Fetal calf serum
FcRn	Neonatal Fc receptor
FGF1	Fibroblast growth factor 1
FGF2	Fibroblast growth factor 2
FGF7	Fibroblast growth factor 7
FGF9	Fibroblast growth factor 9
FGF10	Fibroblast growth factor 10
FGFR	Fibroblast growth factor receptor
FIT-C	Fluorescein isothiocyanate
Flk1	Fetal liver kinase 1
FOXA2	Forkhead box A2
FOXD1	Forkhead box D1
FOXF1	Forkhead box F1
FSGS	Focal segmental glomerulosclerosis
GABRB3	Gamma-aminobutyric acid (GABA) A receptor, beta 3
GATA3	GATA binding protein 3
GBM	Glomerular basement membrane
GDF3	Growth differentiation factor 3
GDNF	Glial cell derived neurotrophic factor
GFB	Glomerular filtration barrier
GFP	Green fluorescent protein
GFRA1	GDNF family receptor alpha 1
GGT1	Gamma-glutamyltransferase 1
GLEPP1	Glomerular epithelial protein 1
GPCRs	G-protein coupled receptors
GSK3β1	Glycogen synthase kinase 3 beta 1
hAD-MSCs	Human adipose derived mesenchymal stem cells
HC	Histochemistry
HDAC	Histone deacetylase

Hes	Hairy enhancer of split
hES	Human embryonic stem cells
Hey	Hes related repressor proteins
HMGA2	High mobility group AT-hook 2
HNA	Human nuclear antigen
HOXB7	Homeo box B7
HOXD11	Homeobox D11
HOXA11	Homeobox DA11
HPRT	Hypoxanthine phosphoribosyltransferase 1
HSCs	Hematopoietic stem cells
HuMt	Human mitochondria
HuNu	Human nuclei antigen
ICC	Immunocytochemistry
IgA	Immunoglobulin A
IGF1	Insulin-like growth factor 1
IGF-R	Insulin-like growth factor 1 receptor
IHC	Immunohistochemistry
IM	Intermediate mesoderm
IMF	Immunofluorescence
iNP	Induced nephron progenitor
iPSCs	Induced pluripotent stem cells
JAG1	Jagged 1
Ki-67	Proliferation-related Ki-67 antigen
KLF15	Kruppel like factor 15
KRT18	Cytokeratin 18
KSCs	Kidney derived stem cells
LHX1	LIM homeobox protein 1
LIF	Leukemia inhibitory factor
LIM1	LIM homeobox 1 (LHX1)
LY294002	2-(4-Morpholinyl)-8-phenyl-1(4H)-benzopyran-4-one hydrochloride
MAFB	Kreisler (Mouse) Maf-related leucine zipper homolog
MAP2	Microtubule-associated protein 2
MCN	Minimal change disease
ME	Mesoderm
MEOX2	Mesenchyme homeobox 2
mES	Mouse embryonic stem cells
MET	Mesenchymal to epithelial transition
mi-RNA	Micro ribonucleic acid
MIXL1	Mix paired-like homeobox
MM	Metanephric mesenchyme
MPP5	Membrane protein palmitoylated 5
MYC	Myelocytomatosis oncogene
mTG	Microbial transglutaminase
MUCIN1	Mucin 1, cell surface associated
MYH11	Myosin heavy chain 11
NANOG	Nanog homeobox
NCAM	Neural cell adhesion molecule 1
N-CDH	N-cadherin
NCK1	Non-catalytic region of tyrosine kinase adaptor protein 1
NCK2	Non-catalytic region of tyrosine kinase adaptor protein 2
NEAA	Non-essential amino acid
NES	Nestin
NICD	Notch intracellular domain
NKX2.5	NK2 transcription factor related, locus 5
NMYC/MYCN	MYCN Proto-oncogene, BHLH transcription factor
NP	Nephron progenitor

NPCs	Nephron progenitor cells
NPHS1	Nephrin
NPHS2	Podocin
NTNG1	Netrin G1
OCT4/POU5F1	Octamer-binding transcription factor 4/POU class 5 homeobox 1
OD	Octadiene
OSR1	Odd-skipped related 1
P	Passage
P27	IFI27 (interferon, alpha-inducible protein 27)
P57	Cyclin-dependent kinase inhibitor 1C
P85	PI3K regulatory subunit alpha
aPKC-PAR3-PAR6	Atypical protein kinase C-partitioning defective 3 homolog-partitioning defective 6 homolog complex
PAX2	Paired box 2
PAX6	Paired box 6
PAX8	Paired box 8
PCR	Polymerase chain reaction
P-CDH	Podocyte cadherin
PECAM1	Platelet and endothelial cell adhesion molecule 1
PenStrep	Penicillin Streptomycin
PFMS	Position frequency matrices
PI3Ks	Phosphoinositide 3-kinases
PIQ	Protein interaction quantitation
PKA	Protein kinase A
PKB	Protein kinase B/Akt
PKCλ/I	Protein kinase C lambda/iota
PNA	Peanut agglutinin
POD1	Podocyte expressed 1
PODXL	Podocalyxin-like protein 1
PS	Primitive streak
PSEN	Presenilin
qRT-PCR	Quantitative real time polymerase chain reaction
RA	Retinoic acid
Rac1	Rac family small GTPase 1
RAF1	v-raf-1 murine leukemia viral oncogene homolog 1
RBP-J	Recombination signal binding protein for immunoglobulin kappa J region
RET	Rearranged during transfection
RFP	Red fluorescent protein
RHO/ROCK	Rho-associated coiled-coil forming protein serine/threonine kinase
RPMI-1640	Roswell Park Memorial Institute (RPMI) 1640
RRT	Renal replacement therapy
RSAD2	Radical S-adenosyl methionine domain-containing protein 2
RTKs	Receptor tyrosine kinases
RUNX2	Runt-related transcription factor 2
SALL1	Spalt like transcription factor 1
SD	Slit diaphragm
SEM	Scanning electron microscope
SIX2	Sine oculis homeobox homolog 1
SIX2	Sine oculis homeobox homolog 2
SLC12A3	Solute carrier family 12 member 3
SLC3A1	Solute carrier family 3 member 1
Slug	Snail family transcriptional repressor 2/SNAI2
SULT1B1	Sulfotransferase family 1B member 1
α-SMA	Alpha smooth muscle actin
SNAI1	Snail family transcriptional repressor 1
SNAI2	Snail family transcriptional repressor 2
SOX1	SRY-Box 1/SRY (sex determining region Y)-box 1

SOX2	SRY-Box 2/SRY (sex determining region Y)-box 2
SOX3	SRY-Box 3/SRY (sex determining region Y)-box 3
SOX17	SRY-Box 17/SRY (sex determining region Y)-box 17
SSEA4	Stage-specific embryonic antigen-4
SULT1B1	Sulfotransferase family 1B member 1
SYNPO	Synaptopodin
T	T-box proteins (Brachyury)
TBX6	T-box 6
TCF	T cell factor
TDGF1	Teratocarcinoma-derived growth factor 1
TEM	Transmission electron microscope
TFBs	Transcription factor binding sites
TFs	Transcription factors
TGFB1	Transforming growth factor, beta 1
TGFB2	Transforming growth factor, beta 2
TIE2	Tyrosine kinase with Ig and EGF homology domains-2
TJP1	Tight junction protein 1
TRA1-1-61	Tumour-related antigen [TRA]-1-60
TRA-1-81	Tumour-related antigen [TRA]-1-81
TRPC5	Transient receptor potential cation channel subfamily C member 5
TRPC6	Transient receptor potential cation channel subfamily C member 6
TUJ1	Neuron-specific class III β-tubulin
UB	Ureteric bud
UE	Ureteric epithelium
VEGF	Vascular endothelial growth factor
VEGFR2	Vascular endothelial growth factor 2
VIM	Vimentin
VPA	Valproic acid
VRAD	Vitamin D3, retinoic, acid and DMEM/F12
WGA lectin	Wheat germ agglutinin
Wnt	Wingless/Integrated
Wnt3a	Wnt family member 3A
Wnt4	Wnt family member 4
Wt	Wild type
WT1	William's tumor 1
Y27632 dihydrochloride	Trans-4-[(1R)-1-aminoethyl]-N-4-pyridinylcyclohexanecarboxamide dihydrochloride
ZO-1	Zona occludin 1

Reference: http://www.genecards.org; https://www.wikigenes.org; Nomenclature: International Union of Pure and Applied Chemistry (IUPAC).