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Reprogramming of mouse renal tubular epithelial cells to induced pluripotent stem cells

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Abstract

Kidney disease has reached epidemic proportions and is associated with high mortality and morbidity rates. Stem cell-based therapy may effectively treat kidney damage by cell transplantation. The breakthrough discovery using a combination of four transcription factors to reprogram genetically somatic cells into induced pluripotent stem (iPS) cells was a milestone in stem cell therapy. The lentivirus was packaged containing OCT4, SOX2, c-MYC and KLF4 transcription factors and then transfected mouse renal tubular epithelial cells (RTECs). The colonies were picked up at 21 days and were tested by cytochemistry, immunofluorescence assay and quantitative real-time polymerase chain reaction. Viral transgene expression levels were also assessed by quantitative analysis. Additionally, embryoid bodies from iPS cells were formed, and immunofluorescence and teratoma assays were performed. Karyotype analysis of mouse RTEC-derived iPS cells was also performed. The iPS cells were indistinguishable from mouse embryonic stem cells with respect to colony morphology, the expression of pluripotency-associated transcription factors and surface markers, embryoid body-mediated differentiation potential and teratoma assays. Quantitative polymerase chain reaction demonstrated that the lentiviral transgenes were largely silenced. The mouse RTEC-derived iPS cells exhibited a normal karyotype of 40,XY. iPS cells can be produced using mouse RTECs, which would be helpful in investigations to ameliorate the symptoms of kidney disease and to slow the progression of kidney disease by *in vitro* and *in vivo* animal studies.

Key Words: *embryonic stem cells, induced pluripotent stem cells, renal tubular epithelial cells, transcription factor*

Introduction

Kidney disease has reached epidemic proportions and is associated with high mortality and morbidity rates. The recovery of renal tissue from acute injury is frequently inadequate because of its complex structure, high metabolism and exposure to waste products and toxins. At the present time, acute kidney injury is mainly treated by dialysis and other supportive therapies. Although dialysis can eliminate toxins and correct water and electrolyte disorders, it cannot improve the reabsorption, endocrine, concentrating and diluting functions of the renal tubules (1). Among surviving patients with long-term acute kidney injury (1–10 years), approximately 19–31% develop chronic kidney disease because of the ineffectiveness of currently available therapies (2). Renal transplantation is the final treatment of choice for patients with end-stage renal disease but has important limitations, including the small number of organ donors and the immune barrier. It is imperative to develop new therapeutic strategies for tissue repair.

An approach that has sparked great interest and gained enormous popularity in recent years is cell-based therapy, which involves cell transplantation to alleviate kidney damage. Because cell therapy has become a new focal point in the regeneration of kidney damage, different sources of stem cells have been investigated. As a progenitor population, stem cells are versatile and multipotent cells and may be isolated from several tissues and expanded *in vitro* (3). Because stem cells (as progenitors) occupy specific sites and most tissues have unique properties, selecting the appropriate cells to deliver to the affected organs or tissues could prove to be a challenge (4). The use of kidney tissue-specific stem cells has been realized. Although several studies have suggested the existence of stem cells in adult renal tissues, including the identification of progenitors in Bowman capsule that are able to replace podocytes

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in vivo (5–8), to date, it has been difficult to confirm the location of progenitor cells within the kidney (9).

A more recent breakthrough discovery provided a milestone in stem cell research by using the overexpression of several transcription factors, including OCT4, SOX2, NANOG, c-MYC, KLF4 and LIN28, for the genetic reprogramming of somatic cells into induced pluripotent stem (iPS) cells; this achievement provides new opportunities in regenerative medicine (10,11). Pluripotent stem cells have the unique ability to differentiate into all cell types of the body. At the present time, some groups have demonstrated that kidney-derived iPS cells can be generated from human renal mesangial and tubular cells (12–14). Another study suggested that disease-specific iPS cells were generated from kidney transplant recipients with a history of autosomal-dominant polycystic kidney disease, systemic lupus erythematosus or Wilms tumor and end-stage renal disease by reprogramming of skin-derived keratinocytes (15). However, it is unclear whether iPS cells can be directly generated from the terminally differentiated kidney cells of other species. In this study, we constructed iPS cells generated from mouse renal tubular epithelial cells (mouse RTECs) co-transfected with the lentivirus-mediated transcription factors OCT4, SOX2, c-MYC and KLF4.

Methods

Lentiviral transduction of mouse RTECs and reprogramming culture

Mouse RTECs (ScienCell Research Laboratories, Carlsbad, CA, USA) isolated from neonate day 2 C57BL/6 mouse kidneys were cultured in epithelial cell medium consisting 500 mL of basal medium, 10 mL of fetal bovine serum (ScienCell Research Laboratories), and 5 mL of epithelial cell growth supplement for animals (ScienCell Research Laboratories). Lentiviral vectors carrying the genes for green fluorescent protein and the reprogramming factors (OCT4, SOX2, KLF4 and c-MYC) were constructed and packaged, and the virus titers were measured according to a previous report (16). Approximately 5×10^4 mouse RTECs were transduced with lentivirus for 12 h. The transduced mouse RTECs were cultured for 6 days in a standard culture medium containing Dulbecco's modified Eagle medium (DMEM)/F-12 (DMEM nutrient mixture F-12 = 1: 1; Invitrogen, Carlsbad, California, USA) and 10% fetal bovine serum (Invitrogen) and passaged onto a feeder layer of mouse embryonic fibroblast cells in an embryonic stem cell-supportive medium containing DMEM/F-12 (DMEM nutrient mixture F-12 = 1: 1; Invitrogen), 15% KnockOut Serum Replacement (Invitrogen), 0.1 mmol/L L-glutamine (Sigma-Aldrich, St Louis, MO, USA), 1% NEAA (Sigma-Aldrich), 0.1 mmol/L β-mercaptoethanol (Sigma-Aldrich) and 1000 units/mL leukemia inhibitory factor (LIF, EMD Millipore, a division of Merck KGaA, Darmstadt, Germany). After 3 weeks, the mouse iPS cell colonies were selected and dissociated by trypsin digestion, and the following experiment was performed. The reprogramming efficiency was approximately 0.1%. For the following experiment, we present the details using one of the colonies.

Differentiation of mouse RTEC-derived iPS cells in vitro

To determine the *in vitro* differentiation ability of the mouse RTEC-derived iPS cells, floating cultivation was used to form embryoid bodies (EBs), as described previously (17). The mouse RTEC-derived iPS cells were dissociated by 0.05% trypsin-ethylenediamine tetraacetic acid and transferred to low-attachment culture dishes (Corning Inc, Acton, MA, USA) in embryonic stem (ES) cell medium. After the suspension culture, EBs were plated onto gelatin-coated, eight-well glass chamber slides, and the cells were fixed for immunocytochemistry analysis after 1–2 weeks.

Cytochemistry and immunofluorescence assay

To examine alkaline phosphatase activity, the iPS cells were processed according to the alkaline phosphatase staining protocol. Briefly, the iPS cells were fixed with 4% paraformaldehyde for 30 min, washed twice with phosphate-buffered saline (PBS) and stained with BCIP/NBT (Sigma-Aldrich) for 15–30 min at room temperature. For the immunofluorescence assay, the undifferentiated iPS cells and their differentiated derivatives were grown on plastic cover slide chambers and treated with cold acetone for 30 min at 4°C and then incubated in blocking solution (1% bovine serum albumin in PBS) for 20 min. The samples were incubated with the following mouse primary antibodies overnight at 4°C: anti-FGF-4 (1:200, MAB5846; R&D Systems, Minneapolis, MN, USA), anti-NANOG (1:100, ab80892; Abcam, Cambridge, UK), anti-OCT4 (1:250, ab19857; Abcam), anti-REX-1 (1:1500, MAB4316; EMD Millipore, a division of Merck KGaA), anti-SSEA-1 (1:10, ab16285; Abcam, Cambridge, UK), anti-alpha fetoprotein (AFP; 1:100, sc-8108; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-desmin (1:200, NB120-15200; Novus Biologicals, Littleton, CO, USA) and anti-nestin (1:150, MAB353; EMD Millipore, a division of Merck KGaA). On the next day, the cells were rinsed three