Xenobiotic Kidney Organogenesis from Human Mesenchymal Stem Cells Using a Growing Rodent Embryo

Takashi Yokoo,*† Akira Fukui,*‡ Toya Ohashi,† Yoichi Miyazaki,* Yasunori Utsunomiya,* Tetsuya Kawamura,* Tatsuo Hosoya,* Masataka Okabe,‡ and Eiji Kobayashi§

*Division of Nephrology and Hypertension, Departments of *Internal Medicine, †Gene Therapy, and ‡Development and Morphogenesis, Institute of DNA Medicine, The Jikei University School of Medicine, Tokyo, and §Division of Organ Replacement Research, Center for Molecular Medicine, Jichi Medical School, Tochigi, Japan*

Given the limits of allogenic organ transplantation, an ultimate therapeutic solution is to establish a self-organ from autologous stem cells and transplant them as syngrafts back into donor patients. It was reported previously that human mesenchymal stem cells (hMSC) that are cultivated in growing rodent embryos can differentiate within a spatially and temporally appropriate developmental milieu, facilitating the first step of nephrogenesis. As another step toward clinical application, the system was modified for progression to complete functional organogenesis. Rat embryos (E11.5) were isolated from uteri, and bone marrow–derived hMSC, which were transfected adenovirally with glial cell line–derived neurotrophic factor and retrovirally with LacZ, were implanted into the nephrogenic site. Forty-eight hours later, ureteric buds were elongated and initial branching was completed. The metanephroi were dissected out, developed further using *in vitro* organ culture for 24 h, transplanted into the omentum of a uninephrectomized rat, and grown for 2 wk. They enlarged and exhibited normal kidney structure and ultrastructure. hMSC-derived LacZ-positive cells were identified throughout the regenerated kidney and were morphologically identical to resident renal cells. Transplantation of developing metanephroi into the LacZ transgenic rat revealed that neo-kidney vasculature originated from the host circulation. Finally, fluid was collected from expanded ureters, and urea nitrogen and creatinine were measured. Levels were much higher in these fluids compared with transplanted rat sera (840.3 ± 184.6 *versus* 30.4 ± 10.8 and 10.1 ± 3.1 *versus* 0.3 ± 0.2 mg, respectively), suggesting that the neo-kidney may produce urine. Taken together, these findings suggest that hMSC can differentiate into a mature renal structure with the potential to replace lost kidney function.

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After recent progress in the field of stem cell research, the potential for tissue regeneration as a replacement for affected tissues has expanded. In nephrology, kidney function may be replaced by dialysis and renal allotransplantation; however, these are not complete therapies, and patients who have ESRD and are on dialysis continue to experience major medical, social, and economic problems. Also, the lack of availability of suitable transplantable organs has prevented kidney transplantation from becoming a practical solution for most cases of ESRD. Therefore, kidney regeneration has attracted considerable attention as an ultimate therapeutic strategy.

Other groups, including ours, recently reported that bone marrow stem cells can contribute to the formation of kidney cells, including mesangial cells (1,2), tubular epithelial cells (3), and podocytes (4). Recent reports have shown that bone marrow reconstitution may replace injured tubular epithelial cells and maintain kidney function in acute renal failure (5,6), although whether such a strategy is applicable in chronic situations is unclear. The approach might be restricted to situations in which kidney damage is not severe and kidney structure remains intact; it is easy to speculate that in cases of renal stem cell damage or destruction or total loss of renal structure, not enough stem cells or scaffold would remain for renal generation. This scenario most likely is in patients who have end-stage renal damage and are undergoing long-term dialysis. For such patients, the ultimate kidney regeneration might be the development of a functional whole kidney from "self" stem cells; however, the kidney is anatomically complicated, and resident cells communicate with each other to function. Therefore, for stem cells to be used for therapeutic kidney regeneration, individual residential cells should be organized to fulfill their functions. Because of this difference between the kidney and other organs such as heart and vessels, a unique approach is necessary for kidney regeneration.

We previously demonstrated that exogenous human mesenchymal stem cells (hMSC) may differentiate and compose part of the nephron in rodent whole embryo by following the program of kidney organogenesis (7). In this system, hMSC were injected at the site of organogenesis in the growing embryo, which was cultured using a whole embryo culture system,

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Address correspondence to: Dr. Takashi Yokoo, Division of Nephrology and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, 3-25-8 Nishi-Shimbashi, Minato-ku, Tokyo, Japan 105-8461. Phone: +813-3433-1111; Fax: +813-3433-4297; E-mail: tyokoo@jikei.ac.jp

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allowing for the initial step of commitment toward nephrogenesis. After 48 h of whole embryo culture, metanephroi were dissected from embryos and subjected to organ culture for 6 d. Using this "relay culture," we showed that injection of glial cell line–derived neurotrophic factor (GDNF)-transfected hMSC into embryos enables generation of chimeric kidneys. In some cases, entire nephrons are hMSC derived.

These hMSC-derived cells are functional, as demonstrated by their ability to metabolize Gb3 accumulated abnormally in the Fabry mouse, suggesting their therapeutic potency (7). However, because the original system uses organ culture for the final development of the metanephros, only an avascular kidney structure forms under these conditions (8). Therefore, vascular integration from the recipient should be completed to form a functional nephron. We focused on the observation originally made by Hammerman's group (9,10) that whole metanephroi that are implanted into the omentum may enlarge, become vascularized, and form mature tubules and glomeruli. On the basis of this observation, we rearranged the original relay culture system to progress to complete functional kidney units. In this study, we show that hMSC may differentiate into functional kidney units, termed "neo-kidney," which may generate urine using this modified relay culture system. The regeneration of affected kidney tissue using this technology may make it possible to treat chronic renal failure.

Materials and Methods

Animals

Wild-type Sprague-Dawley rats and Lewis rats were purchased from Sankyo lab service (Tokyo, Japan). The day on which the vaginal plug was observed at midday was designated as day 0.5. ROSA/LacZ transgenic rats, which express LacZ gene under the control of the ROSA26 promoter (11), were generated as described previously (12). Animals were housed in a positive airflow–ventilated rack and bred and maintained under specific pathogen-free conditions. The following experimental procedures were approved by The Committee for Animal Experiments of The Jikei University.

Culture and Manipulation of hMSC

hMSC were purchased from Cambrex Bio Science, Walkersville, Inc. (Walkersville, MD), and cultured using the manufacturer's recommended conditions. hMSC were used within three cell passages to avoid phenotypic changes. The replication-defective recombinant adenovirus carrying human GDNF cDNA (AxCAhGDNF) was constructed as described before (13), because we previously confirmed that metanephric mesenchyme of E11.5 rat embryo starts to express GDNF to attract the ureteric bud, and this modification significantly increases the number of hMSC-derived nephrons (7). Packaging cells (ψ-crip) that produce a recombinant retrovirus that bears the bacterial LacZ gene, MFG-LacZ, were a gift from Prof. H. Hamada (Department of Molecular Medicine, Sapporo Medical University, Sapporo, Japan). A retroviral vector–containing supernatant was collected from the confluent monolayer of these packaging cells and grown in DMEM with 10% calf serum (Life Technologies/BRL, Gaithersburg, MD). These supernatants (150 ml) were filtered (0.45 μm) and centrifuged at 14000 $\times$ *g* for 16 h at 4°C to concentrate the virus (14), followed by resuspension in conditioned medium (1 ml). hMSC (5.0×10^4/ml culture medium) were cultured with freshly prepared retroviral vector–containing supernatant and polybrene (8 μg/ml) for 2 h. These cells were infected further with AxCAhGDNF (multiplicity of infection = 100).

Modified Relay Culture System and Microinjection of Mesenchymal Cells

Whole embryos were cultured *in vitro* essentially according to the method described previously (15) with several modifications. At E11.5, the uterus was dissected out from anesthetized pregnant rats under a surgical microscope using low-power magnification and freed from the uterine wall, deciduus, and outside membrane layer including Reichert's membrane. hMSC, manipulated as described, were injected using micropipettes. Injections were made into the intermediate mesoderm between the somite and the lateral plate at the level of somite 29, where we previously confirmed that hMSC differentiate into kidney structure without inducing ectopic ureteric bud (7). The chorioallantoic placenta was left intact.

Cultivation of all successfully injected embryos was begun immediately in 15-ml culture bottles that contained 3 ml of culture medium. Medium consisted of 100% immediately centrifuged rat serum supplemented with glucose (2 mg/ml), penicillin G (100 U/ml), streptomycin (100 μg/ml), and amphotericin B (0.25 μg/ml), using the system of rotating bottles (Model RKI10-0310; Ikemoto Co., Tokyo, Japan) to supply the culture with a continuous oxygen flow over the entire culture period. On the basis of experience with successful experiments, we determined the optimal gas exchange schedules for rat embryo as described previously (7).

Metanephric Organ Culture and Implantation

After whole embryo culture, embryos were assessed for heartbeat, whole body blood circulation, and general morphology. Kidney rudiments were dissected under a dissecting microscope and cultured on a 12-mm-diameter, 0.4-μm Nucleopore filter (Corning-Costar, Cambridge, MA) at an air-fluid interface in DMEM supplemented with 20% FBS (Life Technologies BRL), 110 mg/L sodium pyruvate, and 0.5$\times$ streptomycin/penicillin (Life Technologies). After metanephric organ culture for 24 h, kidney anlagen were implanted in the omentum of anesthetized 10- to 15-wk-old male (host) rats. During the same surgery, host rats had the right kidney removed. A flow diagram of the experimental protocol is shown in Figure 1.

Histologic Analysis

Each specimen was fixed in 10% buffered formaldehyde and embedded in paraffin or quickly frozen in liquid nitrogen and embedded in OCT compound. Cryostat sections (6 μm) were dried and fixed with acetone for 15 min. After blocking endogenous biotin with an avidin/biotin blocking kit (Vector Laboratories, Burlingame, CA) and endogenous peroxidase activity with 0.3% H_2O_2 in methanol, we treated the sections with normal goat serum (Vector Laboratories) followed by incubation with the primary antibodies (Ab) overnight at 4°C in a humid chamber. Dilution of primary Ab was 1:4000 for WT-1 (Santa Cruz Biotechnology), 1:250 for desmin (Monosan, Uden, Netherlands), and 8:100 for synaptopodin (Progen Biotechnik GmbH, Heidelberg Germany). After a PBS wash, the sections were incubated with biotinylated anti-rabbit IgG (Vectastain Elite ABC kit, rabbit; Vector Laboratories) for the staining of WT-1 and desmin or biotinylated anti-mouse IgG (Vectastain Elite ABC kit, mouse) for synaptopodin, rinsed in PBS, and incubated with an avidin-biotin-peroxidase complex (Vectastain Elite ABC). The peroxidase was developed with a diaminobenzidine substrate solution (peroxidase substrate kit; Vector Laboratories). All kits were used according to manufacturer instructions. X-gal assay was

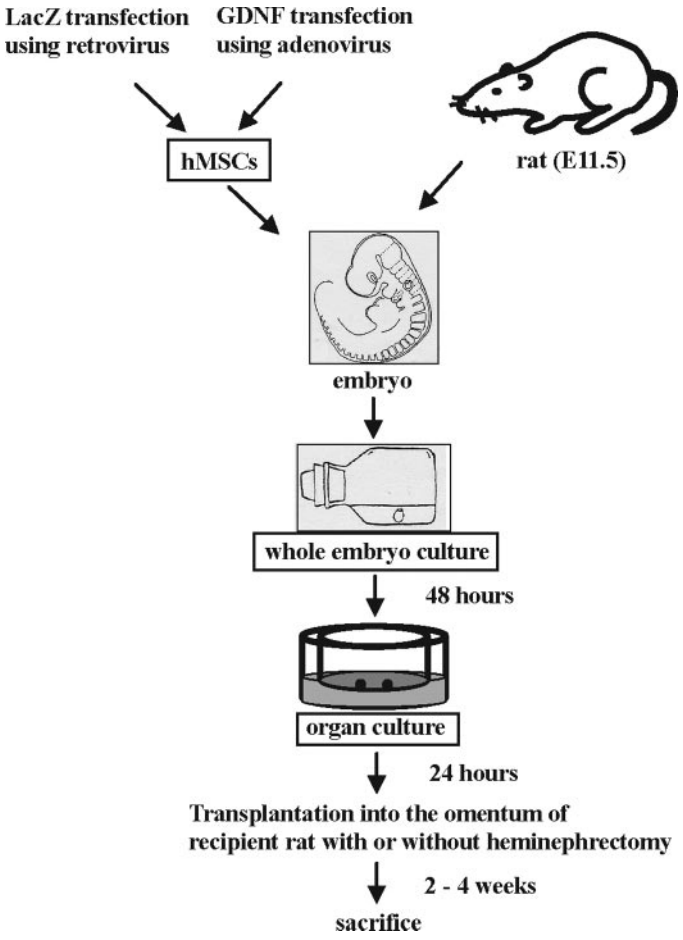

Figure 1. A flow diagram of the modified relay culture system.

performed to assess the expression of the LacZ gene as described previously (16).

Electron Microscopy

For electron microscopy, neo-kidney was doubly fixed with 2% glutaraldehyde/0.1 M sodium phosphate (pH 7.2) and 1% osmium tetroxide/0.1 M sodium phosphate (pH 7.2) and dehydrated with a grade series of ethanol. Samples then were embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and observed with a Hitachi H-7500 transmission electron microscope (Hitachi, Tokyo, Japan).

FACS-Gal Assay

Metanephroi that were regenerated by relay culture were digested in 500 μl of collagenase type I (1 mg/ml; Sigma, St. Louis, MO) for 30 min at 37°C. DMEM with 10% FBS then was added, and cells were pelleted. The cellular digests were filtered through a double layer of sterile 40-μm nylon mesh and labeled with fluorescein digalactoside (Molecular Probes, Eugene, OR) using transient permeabilization by hypotonic shock as essentially described by Fiering *et al.* (17). LacZ-positive cells were collected using a cell sorter (Becton Dickinson, Franklin Lakes, NJ).

Reverse Transcription–PCR

Total RNA was extracted from sorted LacZ-positive cells with the RNeasy mini kit (Qiagen GmbH, Hilden, Germany), and cDNA was synthesized using SuperScript II Reverse Transcriptase (Life Technologies BRL, Rockville, MD) following manufacturers' protocols. The expression of aquaporin-1, parathyroid hormone (PTH) receptor 1, 1α hydroxylase, nephrin, glomerular epithelial protein 1, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and platelet-endothelial cell adhesion molecule-1 were assessed by amplified products after PCR. The primer sequences and reaction conditions are listed in Table 1.

Statistical Analyses

Data are expressed as the means ± SEM. Statistical analysis was performed using the two-sample t test to compare data between different groups. $P < 0.05$ was considered significant.

Results

Modification of the Relay Culture System

Metanephroi that were isolated from different stages of rat embryos were transplanted into the omentum of recipient rats. As originally reported (18), we confirmed that heminephrectomy at the time of transplantation may accelerate the development of transplanted metanephros (data not shown). After 2 wk, recipients were killed, and development of the metanephroi was examined. As shown in Figure 2a, metanephroi that were isolated from embryos that were older than E13.5 may develop in the omentum, whereas no hint of a metanephros was found in the omentum in which metanephroi from embryos that were younger than E12.5 had been transplanted. Histologic analysis revealed that developed metanephroi from E13.5 embryos within the omentum maintained the renal structure (Figure 2b); in addition, desmin-positive mesangial cells and WT-1– and synaptopodin-positive podocytes were detected in the glomeruli (Figure 2c), suggesting that developed metanephroi were well differentiated and structurally mature. Twenty-four hours of incubation in the organ culture system was enough for development to a size identical to that of the E13.5 metanephroi; therefore, the original relay culture system was adjusted, and, after whole embryo culture for 48 h, metanephroi were isolated from growing embryos, incubated for 24 h using the organ culture system, and transplanted into the omentum of recipient rats (Figure 1). This system is termed the "modified relay culture system."

Generation of Neo-Kidney from hMSC

After 2 wk in the omentum, the kidney rudiment grew up to 64 ± 21 mg (Figure 3a). Developed kidneys that were subjected to X-gal assay and paraffin sections were observed by light microscopy. X-gal–positive cells were identified throughout the metanephric rudiment (Figure 3b) and were morphologically identical to podocyte and tubular epithelial cells. These hMSC-derived LacZ-positive cells were FACS-sorted using the FACS-Gal assay, and their gene expression was analyzed by reverse transcription–PCR. As shown in Figure 3c, podocyte-specific genes (nephrin and glomerular epithelial protein-1) and tubular epithelial cell–specific genes (aquaporin-1, 1α hydroxylase, and PTH receptor-1) were expressed in the LacZ-positive cells. Because we previously showed that during whole embryo culture, hMSC complete an initial step essential for commitment to a renal fate (7), these data suggest that during modified relay

Table 1. PCR primers[a]

Primer	Base Sequence	Fragment Length (bp)	OAT (°C)
Human 1α hydroxylase sense	CCTGAACAACGTAGTCTGCG	620	60
Human 1α hydroxylase antisense	CAGCTGTGATCTCTGAGTGG		
Rat ICAM-1 sense	CTGGAGAGCACAAACAGCAGAG	385	55
Rat ICAM-1 antisense	AAGGCCGCAGAGCAAAAGAAGC		
Rat VCAM-1 sense	TAAGTTACACAGCAGTCAAATGGA	283	50
Rat VCAM-1 antisense	CACATACATAAATGCCGGAATCTT		
Rat PECAM-1 sense	AGGGCTCATTGCGGTGGTTGTCAT	348	52
Rat PECAM-1 antisense	TAAGGGTGCCTTCCGTTCTAGAGT		
Human AQP-1 sense	CTTGGACACCTCCTGGCTATTGAC	625	60
Human AQP-1 antisense	AGCAGGTGGGTCCCTTTCTTTCAC		
Human PTHr sense	GATGCAGATGACGTCATGAC	482	58
Human PTHr antisense	CAGGCGGTCAAACACCTCCCG		
Human GLEPP-1 sense	TCACTGTGGAGATGATTTCAGAGG	74	58
Human GLEPP-1 antisense	CGTCAGCATAGTTGATCCGGA		
Human nephrin sense	CAACTGGGAGAGACTGGGAGAA	188	58
Human nephrin antisense	AATCTGACAACAAGACGGAGCA		
Human β-MG sense	CAGGTTTACTCACGTCATCCAGC	235	[b]
Human β-MG antisense	TCACATGGTTCACACGGCAGG		
Rat GAPDH sense	CATCAACGACCCCTTCATT	197	[b]
Rat GAPDH antisense	ACTCCAGCACATACTCAGCAC		

[a]AQP, aquaporin; GLEPP, glomerular epithelial protein; ICAM, intercellular adhesion molecule; MG, microglobulin; OAT, optimum annealing temperature; PECAM, platelet-endothelial cell adhesion molecule; PTHr, parathyroid hormone receptor; VCAM, vascular cellular adhesion molecule.

[b]For human MG and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH), two-step amplification (1 min at 94°C and 1 min at 66°C, 43 cycles) were applied. PCR conditions were as follows: 10 min at 95°C and 45 s at 94°C, I minat the OAT, and 1 min at 72°C, 36 cycles; 10 min at 72°C.

culture, they further develop to become the mature kidney structure.

Origin of the Vasculature in the Neo-Kidney

For examination of the origin of the vasculature in the neo-kidney, LacZ transgenic rats were generated and used as recipients of the modified relay culture system so that tissues that are derived from the recipient may be distinguished from those of the donor by X-gal assay. This transgenic rat was confirmed to express the LacZ gene strongly in the peritubular capillaries (data not shown). After the modified relay culture, developed metanephroi were dissected out from the omentum and observed under stereoscopic microscope. Several vessels from the omentum were integrated into neo-kidney (Figure 4a), and X-gal assay of this neo-kidney showed that most of peritubular capillaries were LacZ positive (Figure 4b), suggesting that they were of recipient origin. Reverse transcription–PCR of FACS-sorted cells revealed that LacZ-positive cells expressed endothelial cell–specific genes (intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and platelet-endothelial cell adhesion molecule-1; Figure 4d). Furthermore, electron microscopic analysis revealed that red blood cells are located in the glomerular vasculature (Figure 4c). These data suggest that vasculature of the neo-kidney in the omentum originates from the host and communicates with the host circulation. Of note, well-differentiated podocyte structures with slit membranes were developed, suggesting that this glomerulus may function to filter the blood of the recipient.

Production of Urine by the Neo-Kidney

For examination of whether the neo-kidney has the potential to produce urine, neo-kidney was left for 4 wk in the omentum to develop further. As shown in Figure 5, neo-kidneys developed hydronephrosis, which suggests urine production; because the ureter was buried under the fat of the omentum with no egress for the urine, hydronephrosis was the result. The liquid that was retained in the dilated ureter was collected using a mouth pipette, and urea nitrogen and creatinine were measured. Their levels were much higher in these fluids compared with those found in the sera of recipient rat (840.3 ± 184.6 *versus* 30.4 ± 10.8 and 10.1 ± 3.1 *versus* 0.3 ± 0.2 mg, respectively; $P < 0.05$), which was comparable with native urine. Taken together, these findings suggest that hMSC can differentiate into mature neo-kidney, which has the potential to produce urine.

Discussion

Therapeutic kidney regeneration has been the focus of many researchers as an ultimate therapy, and some types of acute renal failure are reportedly treatable by transplantation of tissue- or bone-marrow–derived renal stem cells (5,19). The basis

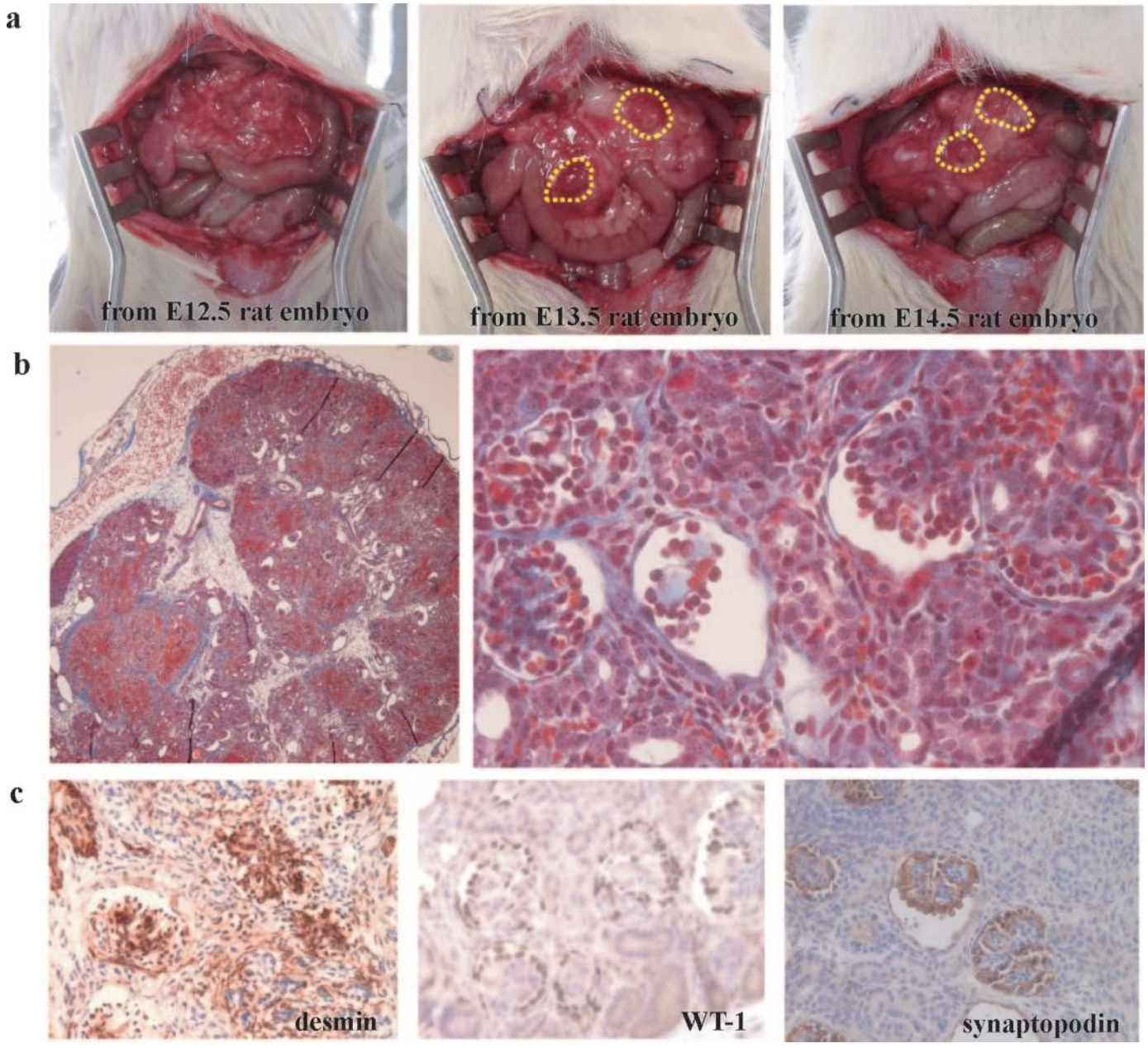

Figure 2. In vivo development of kidney primordia in the omentum. Kidney primordia from different stages of rat embryo were implanted into the omentum of host rats. After 2 wk, host rats were killed and examined to determine whether the implants had developed (a). Yellow dotted lines indicate the outline of the developed kidney. Kidney primordia from an E13.5 rat embryo that had grown in the omentum were subjected to Masson staining and shown in low (left) and high (right) magnification (b). To confirm that they had differentiated into mature structures, the expression of desmin, WT-1, and synaptopodin, which are expressed in well-differentiated mesangial cells and podocytes, was assessed by immunostaining (c). Original magnifications: ×100 in b, left; ×400 in b, right, ×400 in c.

of these approaches is the idea that, in some circumstances, kidney probably cannot regenerate itself but may achieve regeneration with a supply of renal stem cells of exogenous origin. However, cellular transplantation-based regeneration may not be applied in cases in which renal structure has been destroyed, which may be the case in most patients with chronic renal failure. Therefore, our focus is on the patients who already have ESRD, and we have striven to establish a system of kidney regeneration that resets the abolished function by replacing damaged renal structures, including the scaffold. We previously found using our original relay culture system that hMSC achieve the initial step for nephrogenesis in the growing embryo (7). In our study, this system was combined with a "growing kidney *in situ* system" (9,10) to develop further kidney anlagen. This modification may result in hMSC becoming functional neo-kidney, vascularized by in-growth of recipient

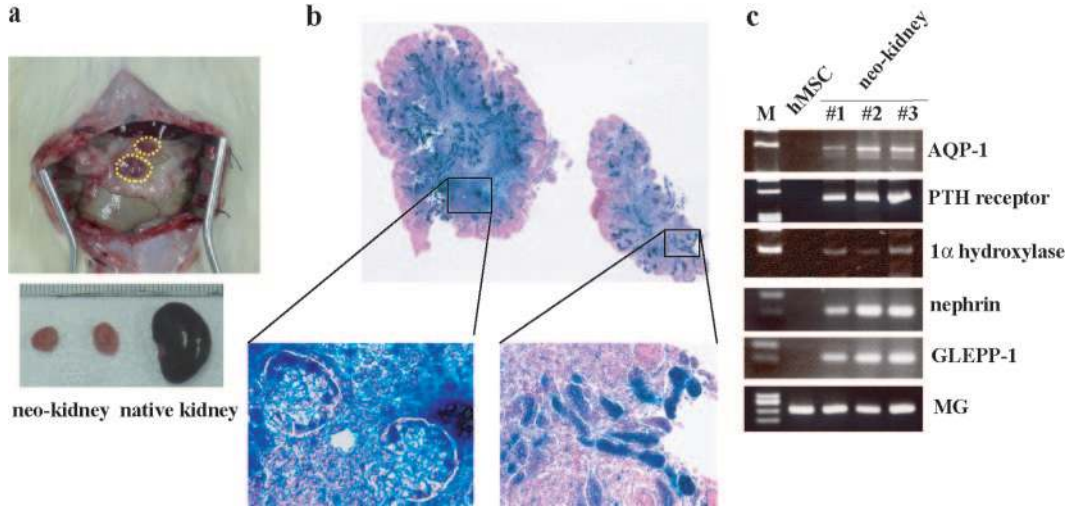

Figure 3. Generation of neo-kidney from human mesenchymal stem cells (hMSC). LacZ-positive hMSC were differentiated *in vivo* using the modified relay culture system (a). Yellow dotted lines indicate the outline of the developed kidney in the omentum (top). These were dissected out and compared with native kidney (bottom). The resulting neo-kidneys were subjected to an X-gal assay to trace the transplanted hMSC (b, top). The morphology of these LacZ-positive cells (shown under high magnification, bottom) and the renal structures to which they contributed was consistent with their being podocytes (left) and tubular epithelial cells (right). LacZ-positive cells from the neo-kidney were sorted and subjected to reverse transcription–PCR (RT-PCR) for expression analysis of aquaporin-1 (AQP-1), parathyroid hormone (PTH) receptor 1, 1α hydroxylase, nephrin, glomerular epithelial protein (GLEPP-1), and human-specific β_2 microglobulin (MG) (c). Lane 1, marker (ϕX174/HaeIII); lane 2, hMSC; lanes 3 through 5, neo-kidney from three individual experiments. Representative photographs are shown. Magnification, ×400 in b, bottom.

blood vessels and able to produce urine. Current surgical skills are not sufficient to achieve ureteroureterostomy between the ureter from neo-kidney and native ureter in rat; however, it reportedly is possible with an extremely skilled hand at rodent surgery with the result that the transplanted metanephros secretes a concentrated urine and the lifespan of the anephric rat is prolonged (20). With application of this technical development, our approach possibly could lead to long-term renal replacement therapy in the future.

Xenotransplantation of developing renal anlagen to replace renal function of damaged kidney is being pursued in several laboratories (21). It has been reported that transplantation of developing kidneys might be advantageous relative to developed kidneys because (*1*) antigen-presenting cells would be absent from the renal primordium, having not yet developed in the donor or migrated into the metanephros (22,23); (*2*) donor antigens such as MHC may not be expressed on renal anlagen to the extent that they are expressed in developed kidney (24,25); (*3*) the T helper 2–biased immune response to transplanted fetal tissue differs from the response of adult tissue (26); and (*4*) the endothelial lining of its blood vessels, which are exposed directly to components of the immune system of the host, may originate from the host's circulation (10).

In fact, metanephroi can mature after implantation into the anterior eye chamber (27), beneath the renal capsule (28), into tunnels fashioned in the renal cortices (29), and into the omentum (9,10) of host animals. Among these implantation sites, omentum may be the most clinically practical because it is not confined by a tight organ capsule and there are little or no space limitations on transplant growth. Furthermore, it may be easily accessible using endoscopic surgery in the clinical setting. Therefore, we chose to transplant the renal anlagen into the omentum and adjusted our previous system with this xenotransplantation system. Although it has been reported that immunosuppression should be necessary for xenotransplantation of renal anlagen (18), our data suggest that using the modified relay culture system, hMSC may differentiate and develop the renal unit in the rodent environment without immunosuppressive drugs. Although currently we cannot explain the mechanism, it is worth speculating that the rodent embryo may facilitate immunotolerance for both self and xenogenic cells if the xenogens are transplanted before the host's immune system has been developed. Further examination is necessary to address this speculation.

hMSC that were found in adult bone marrow were shown recently to retain plasticity and to differentiate into several different cell types, depending on their microenvironment (30). Embryonic stem (ES) cells are another option as an origin of kidney regeneration (31). Unlike hMSC (7), ES cells that are injected into established metanephroi may be integrated into the renal structure during organ culturing (32), suggesting that ES cells are feasible for forming renal structure compared with MSC. We previously found that hMSC express Six1, Eya1, and Sall1 but do not express WT1 and Pax2 (our unpublished data), showing that it has yet to manifest completed "nephrogenic molecular features" at the onset. Although we tried only GDNF for enhancing the chimerism in the neo-kidney, other molecules or combinations of molecules should be examined to seek the optimal condition for neo-generation. In contrast to ES cells, however, adult MSC can be isolated from autologous bone marrow and applied for therapeu-

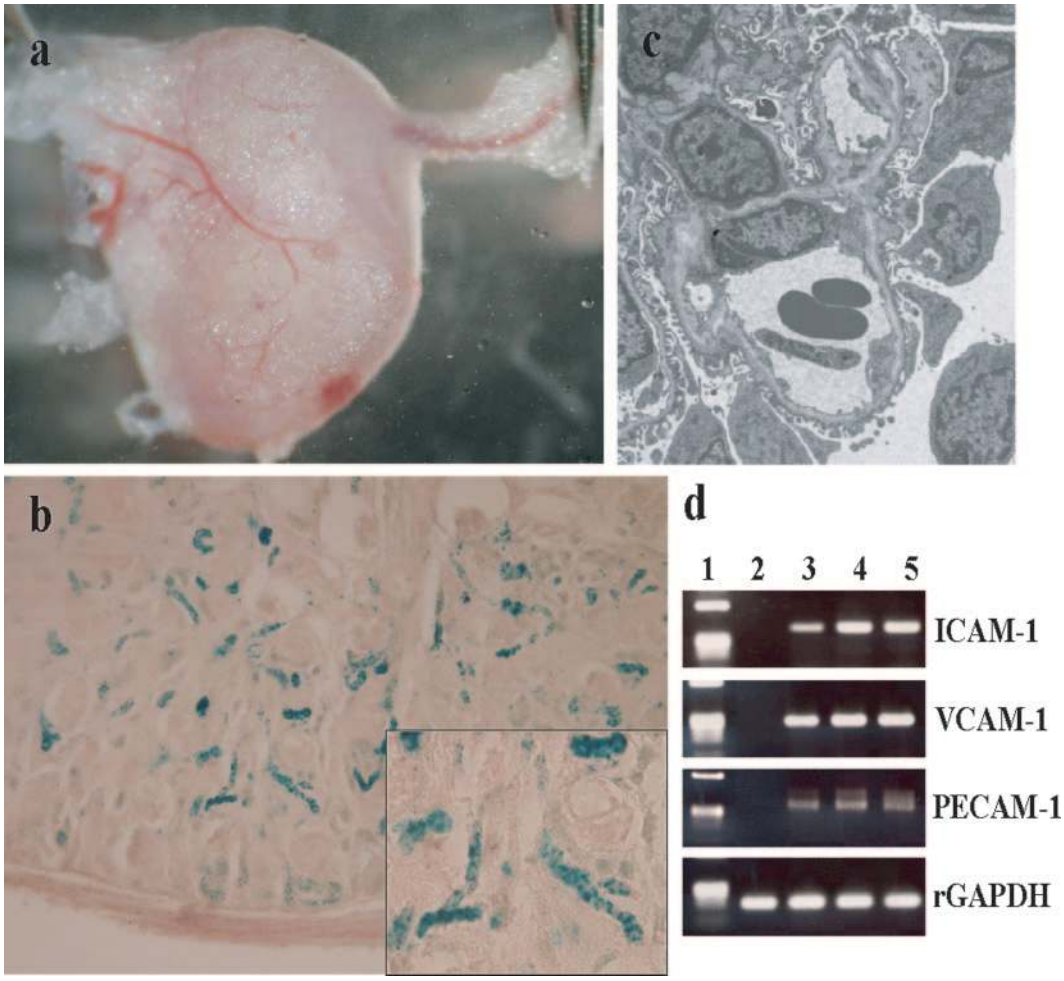

Figure 4. Origin of the vasculature in the neo-kidney. For examination of the origin of the vasculature in the neo-kidney, LacZ transgenic rats were used as hosts at the final step of the modified relay culture. The neo-kidneys were dissected out from the omentum, and their appearance was observed under a stereoscopic microscope (a). They also were subjected to the X-gal assay to trace the host-originated cells (b) and to electron microscopic analysis (c; arrows indicate red blood cells). After modified relay culture, the resulting kidney primordia were digested, and single cells were subjected to the FACS-gal assay. LacZ-positive cells were sorted and subjected to RT-PCR for expression analysis of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), platelet-endothelial cell adhesion molecule (PECAM-1), and rat glyceraldehyde-3-phosphate dehydrogenase (rGAPDH; d). Lane 1, marker (ϕX174/HaeIII); lane 2, kidney primordia before implanting in omentum; lanes 3 through 5, neo-kidney from three individual experiments. Representative photographs are shown. Magnifications: ×100 in b, ×6000 in c.

tic use without any serious ethical issues or immunologic consequences. We believe that MSC are more practical compared with ES cells for therapeutic renal regeneration; therefore, primary hMSC that were obtained from the bone marrow of healthy volunteers were used throughout this study.

Our current system could not exchange the Wolffian duct for one of human origin; collecting duct to ureter was built up by the host embryo tissue. As described before, the developing renal anlagen are less immunogenic compared with developed kidney; however, even a small contamination of such chimeric cells might evoke an unexpectedly enormous immunoreaction. Therefore, we currently attempt to overcome this hurdle by two different approaches: (*1*) eliminating xenogenic cells before transplantation into the omentum using a transgenic host that carries a regulated suicide gene and (*2*) exchanging the posterior part of the Wolffian duct to human during its elongation so that the collecting duct in the neo-kidney may be of host origin.

Furthermore, kidney plays a role locally in removing uremic toxins and excess fluid by producing urine and also systemically contributes to the maintenance of homeostasis through hematopoiesis, BP control, and calcium/phosphorus balance. Therefore, secreting proteins (erythropoietin and renin), receptors (PTH receptor), and processing enzymes (1α hydroxylase) of the established organ should be of host origin to integrate properly into the host endocrine system. As examples, we examined the nucleotide sequences of 1α hydroxylase and PTH receptor-1 from RNA that was extracted from neo-kidney and confirmed that they contained human-specific products (data not shown), suggesting that neo-kidney may produce human protein and be integrated in human homeostasis. In this regard,

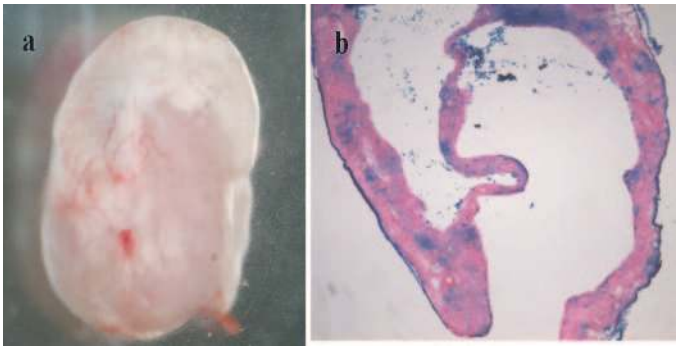

Figure 5. Production of urine by the neo-kidney. For examination of whether the neo-kidney has the potential to produce urine, neo-kidney was left for 4 wk in the omentum to develop further. The appearance of the resulting neo-kidney was observed under a stereoscopic microscope (a), and histologic analysis after the X-gal assay is shown (b). Of note, the neo-kidney developed hydronephrosis, suggesting that urine was produced, leading to a dilated ureter.

we believe that this neo-kidney, even in its current chimeric form, has a substantial advantage over the xeno-metanephros.

In addition, we found that neo-kidney may enlarge up to 64 ± 21 mg, which is comparable to a native rat kidney. Even though the neo-kidney does not need to be the same size as the native kidney for relief from dialysis, this size is too small for human renal function, and we need to seek larger host embryos to establish larger organs that are more suited for use in humans. It was reported recently that pig metanephroi that are transplanted into rat omentum may develop a larger volume (diameter and weight) than a normal rat kidney (33). Although there are a few animals whose renal developmental processes are fully understood, we need a larger animal that can be applicable to our system.

Conclusion

We have demonstrated a system that might provide the means to generate self-organs from autologous MSC by using inherent developmental and angiogenic systems. We have only just begun this research but still believe that advances in technology, such as the culturing systems, may shift the paradigm from "re"-generative to "neo"-generative medicine, with the potential for extensive therapeutic application.

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