Branching ducts similar to mesonephric ducts or ureteric buds in teratomas originating from mouse embryonic stem cells

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As NH nephrons are highly differentiated structures, and once
branching morphology; mesonephric nephrons; renal primordia; de-
velopment; kidney regeneration

RENAL NEPHRONS ARE HIGHLY DIFFERENTIATED STRUCTURES, AND ONCE DAMAGED, THEY RARELY RECOVER THEIR FUNCTION. RENAL TUBULES REGENERATE IN THE CASE OF ACUTE TUBULAR NECROSIS (12), BUT THE GLOMERULI DO NOT IN MANY KIDNEY DISEASES. THIS FAILURE TO FULLY REGENERATE GIVES RISE TO PATIENTS WITH END-STATE RENAL FAILURE. DUE TO A LACK OF DONORS, ONLY A LIMITED NUMBER OF SUCH PATIENTS CAN RECEIVE KIDNEY TRANSPLANTS, AND MOST MUST UNDERGO DIALYSIS FOR THE REMAINDER OF THEIR LIVES. DESPITE OUTSTANDING PROGRESS IN REGENERATIVE MEDICINE AND TISSUE ENGINEERING, NO MEANS TO REGENERATE KIDNEY TISSUES HAVE BEEN ESTABLISHED. THERE IS A NEED, THEREFORE, TO DEVELOP REGENERATIVE MEDICINE FOR THE KIDNEY.

Nephrogenesis is initiated and continued as a result of reciprocal inductive interaction between two primordial tissues, namely, the metanephric mesenchyme and the ureteric bud (36). First, the ureteric bud grows out from the mesonephric duct into the metanephric mesenchyme. When these two elements come into contact, the ureteric bud undergoes branching morphogenesis, generating much of the collecting duct system. Meanwhile, the metanephric mesenchyme undergoes nephrogenesis (36). Control genes involved in the early stages of metanephrogenesis have been elucidated (19). Knockout studies have resulted in a lack of metanephric kidney, showing that these control genes are essential for kidney development. In brief, Pax-2 (44), Lim-1 (39), c-Ret (6, 37), and Emx2 (22) are expressed in the invading ureteric bud, whereas Sall1 (25), WT-1 (18), Eya-1 (46), GDNF (23, 29, 35), and Wnt-4 (41) are expressed in the metanephric mesenchyme. Pax-2 is also expressed in induced mesenchymal cells (44).

Recent progress in embryonic stem (ES) cell technology for regenerative medicine has been significant. ES cells derived from the inner cell mass of blastocyst-stage early mammalian embryos (5, 7) can proliferate indefinitely and differentiate into derivatives of all three primary germ layers in vitro (24). The pluripotency of mouse ES cells has been demonstrated in knockout mice in which ES cells integrated into early embryos produce all cell lineages in the chimeras. Except for these cases of integration into ontogenic processes, the cell lineages induced from ES cells have been restricted. For ES cell-derived kidney cells and structures, limited information is available. Thomson et al. (43) reported the induction of mesonephric nephron-like structures in teratoma tissues originating from human ES cells. This is a noteworthy step in the ability to induce regeneration of the nephron, the functional unit of the kidney. However, it is not certain whether the ES cell-derived nephrons belonged to the mesonephros or the metanephros. It is also not clear whether the collecting duct system was simultaneously induced. The ureteric bud and its origin, the mesonephric duct, are a key element in the induction of metanephric nephrogenesis.

Teratoma formation, particularly in vivo, is a useful experimental model for surveying the potency of ES cells because it allows various cell lineages to develop with three-dimensional architecture. To gain access to kidney tissue regeneration, in the present study we searched for renal primordial structures originating from mouse ES cells in vitro and in vivo. Gene expression essential for metanephrogenesis was investigated in embryoid body (EB) outgrowths and in teratomas originating...
from ES cells. In addition, histochemical detection of renal primordial structures was carried out in the teratoma tissues. In this report, we describe the potency of mouse ES cells to generate the primordium of the collecting duct system, that is, the mesonephric duct and ureteric bud.

**MATERIALS AND METHODS**

**Cells and animals.** Three lines of mouse ES cells were used in the study. ES cell line R1 was a gift from Dr. Andras Nagy. ES cell line D3, developed by Doetschman et al. (5), was obtained from the American Type Culture Collection (Manassas, VA) and previously

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**Table 1. Antibodies and lectin used and gene expression pattern in kidney development**

<table>
<thead>
<tr>
<th>Antibody/Lectin*</th>
<th>Gene†</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td><strong>Duct system</strong></td>
<td></td>
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</tr>
<tr>
<td>Mesonephric duct</td>
<td>+</td>
<td>12, 17</td>
</tr>
<tr>
<td>Uninduced ureteric bud</td>
<td>+</td>
<td>32, 34</td>
</tr>
<tr>
<td>Duct tip</td>
<td>+</td>
<td>33, 45</td>
</tr>
<tr>
<td>Duct trunk</td>
<td>+</td>
<td>34</td>
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<tr>
<td><strong>Adult kidney</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medullary collecting duct</td>
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<td></td>
</tr>
<tr>
<td>Cortical collecting duct</td>
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<tr>
<td><strong>Nephron</strong></td>
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<tr>
<td>Mesonephric tubule</td>
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</tr>
<tr>
<td>Mesonephric glomerulus</td>
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<td></td>
</tr>
<tr>
<td>Developing metanephros</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninduced mesenchyme</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Induced mesenchyme</td>
<td>+</td>
<td></td>
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<tr>
<td>Vesicle</td>
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<td></td>
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<tr>
<td>Comma-shaped body</td>
<td>+</td>
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<tr>
<td>S-shaped body</td>
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<tr>
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<td>Glomeruli</td>
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<td>Proximal tubule</td>
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<td>Thin limb of Henle’s loop</td>
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<tr>
<td>Distal tubule</td>
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*Data are primarily in accordance with references and are modified based on control findings in the staining condition employed. +, Detectable; –, undetectable; – +, weak reactivity; blank, no information. †Data are based on references and the Kidney Development Database by Davies JA and Brandli AW (http://golgi.ana.ed.ac.uk/kidhome.html). +, Expressed; –, not expressed; blank, no information.

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**Table 2. Primer sets for PCR analysis**

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also binds to the epithelium of ureteric bud branches (20). Antibody for WT-1 (rabbit polyclonal, Santa Cruz Biotechnology, Santa Cruz, CA) was employed to evaluate the induction of metanephric mesenchymal cells (33).

Cell culture. ES cells were cultured in 5% CO2 at 37°C for 72 h in DMEM (GIBCO, Grand Island, NY) supplemented with 20% Knockout SR (GIBCO), 100 μM nonessential amino acids (GIBCO), 1 mM sodium pyruvate (GIBCO), 100 μM β-mercaptoethanol (Sigma, St. Louis, MO), and 10³ units/ml of leukemia inhibitory factor (Chemicon, Temecula, CA) on feeder cells of mitomycin C-inactivated STO fibroblasts (13). To induce EB formation, the cultures were dissociated with 0.05% trypsin-EDTA, and the cells were resuspended in Iscove’s modified Dulbecco’s medium (GIBCO) containing 20% FBS (GIBCO), 100 μM nonessential amino acids, 1 mM sodium pyruvate, and 100 μM β-mercaptoethanol without leukemia inhibitory factor in a concentration of 1,000 cells/50-μl drop, and cultured in 5% CO2 at 37°C for 5 days using the hanging-drop method (21). The EBs formed in the drops were replated onto a gelatin-coated 96-well dish with one EB/well and cultured in the same medium up to 21 days for RT-PCR analysis. EB outgrowths on day 7 of expansion were taken for transplantation to make teratoma tissues.

RNA extraction and RT-PCR analysis. Using an RNaseasy Mini Kit (Qiagen, Valencia, CA), total RNA was extracted from EBs on culture day 5, from the EB outgrowths every 3 days of expansion, and from the teratomas 14 and 28 days after transplantation. DNase-treated total RNA was used for the first-strand cDNA. This reaction was performed using SuperScript II and RNase OUT recombinant ribonuclease inhibitor (Qiagen) in accordance with the manufacturer’s protocol.

Table 3. Gene expression essential for kidney development during ES cell differentiation

<table>
<thead>
<tr>
<th>129sv-ES</th>
<th>EB</th>
<th>3</th>
<th>6</th>
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<th>15</th>
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<th>T14</th>
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Fig. 1. Morphology and RT-PCR analysis of 129/sv-embryonic stem (ES) cell differentiation. Similar patterns of growth, development, and gene expression were seen in all 3 ES cell lines. A: undifferentiated mouse ES cells form dome-shaped colonies on culture day 3. Bar = 70 μm. B: embryoid body (EB) on day 5 of a suspension culture by the hanging-drop method. Bar = 70 μm. C: EB outgrowths on day 7 of expansion. By this stage, differentiation of ES cells into a wide variety of lineages occurred. Bar = 200 μm. D: an expression pattern of genes essential for kidney development during the differentiation of 129sv-ES cells. In this case, Lin-1, Emx2, Sall1, Eya-1, and GDNF are expressed in EBs before expansion (EB), and WT-1 and Wnt-4 expressions are detected in EB outgrowths from day 3 of expansion onward. Pax-2 and c-ret appear on day 6. All these gene expressions are also detected in the transplants 14 days after transplantation (T14). β-Actin served as internal standard. Numbers at the top indicate the day of expansion of EB outgrowths.

used by us (4). The ES cell line developed from 129/sv-strain mouse (129sv-ES) was purchased from Cell and Molecular Technologies (Phillipsburg, NJ). BALB/c nu/nu male mice at postnatal week 5, at least three animals for each survival time in each cell line, were used as hosts. Embryos on embryonic day 12 (E12) and adult ICR-strain mice at postnatal week 6 were used as controls for histochemical staining. All animals were purchased from SLC (Hamamatsu, Japan). The investigation was approved by the Animal Care Committee of Shinshu University.

Antibodies and lectin. For detection of the primordial kidney duct system, antibodies specific for Pax-2 (rabbit polyclonal, Zymed Laboratories, San Francisco, CA), endo A cytokeratin, the mouse homolog of cytokeratin 8 (clone TROMA-1, rat IgG, Developmental Studies Hybridoma Bank, Iowa City, IA), and kidney-specific cadherin (Ksp-cadherin; clone 4H6/F9, mouse IgG1, Zymed Laboratories), and FITC- or biotin-labeled Dolichos biflorus agglutinin (DBA; Vector Laboratories, Burlingame, CA) were employed (Table 1). Pax-2 antibody detects the mesonephric duct and nephron, ureteric bud and its derivatives, induced metanephric mesenchyme, and metanephric nephron at comma- and S-shaped body stages (12, 32–34). Endo A cytokeratin antibody detects the endodermal epithelium and also mesodermal epithelia such as the mesonephric duct and nephron and ureteric bud (17, 34, 45). Ksp-cadherin antibody detects the ureteric bud, mesonephric duct, Müllerian duct, and developing tubules in the mesonephros and metanephros in embryos (11, 38). DBA

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ES, embryonic stem; EB, embryoid body; T14 and T28, transplants 14 and 28 days after transplantation; +, Detectable; –, undetectable; numbers at the top of the columns indicate the day of expansion of EB outgrowths; EB refers to EBs on suspension culture day 5.
cDNA samples were subjected to PCR amplification with specific primers for genes essential for kidney development (Table 1) and for control genes. Amplification consisted of denaturation at 94°C for 3 min followed by 35 cycles, each consisting of denaturation at 94°C for 1 min, annealing at the temperatures specified for each of the primer sets for 1 min, and extension at 72°C for 1 min. Finally, the product was extended at 72°C for 7 min. The PCR primers, annealing temperatures, and size of the amplified products are shown in Table 2.

PCR reaction products were separated on 1.5% agarose gels, stained with ethidium bromide, and visualized with UV light.

**Cell transplantation.** Cultured EB outgrowths on day 7 of expansion were washed with PBS to remove dead cells. After detachment from the culture dish with PBS containing 0.05% trypsin-EDTA, cells were collected in a 15-ml tube and centrifuged at 1,000 rpm for 5 min at 4°C. The cell pellet was resuspended within Iscove’s medium with the same supplements as the procedure at 4°C. The host mice were anesthetized with an injection of Nembutal (0.05 mg/g body wt) dissolved in physiological saline. Cells (1.5 × 10^6) were injected into the retroperitoneum of host mice using a 1.0-ml syringe with a 26-gauge needle.

**Light microscopic histochemistry.** The teratomas derived from injected EB outgrowths cells were excised from the host mice under deep anesthesia with Nembutal 14 and 28 days after transplantation, fixed in 4% paraformaldehyde/0.1 M phosphate buffer, pH 7.4, for 24 h at 4°C, and incubated within a graded concentration of sucrose solution (15, 20, and 30%). Samples were embedded in Tissue Tek OCT compound (Sakura Finetek, Torrance, CA) and frozen rapidly. Cryosections were cut at a thickness of 5 μm in each teratoma by a Cryostat CM1900 (Leica, Heidelberg, Germany) fixed on glass slides. After fixation in methanol for 3 min, specimens were stained with a toluidine blue technology, Bernried, Germany) fixed on glass slides. After fixation in methanol for 3 min, specimens were stained with a toluidine blue compromised with 4',6-diamidino-2-phenylindole dihydrochloride (Molecular Probes), specimens were observed with an Olympus Fluoview confocal laser-scanning microscope equipped with Ar and He/Ne lasers or a Leica LSM TCS SP2 AOBS confocal laser-scanning microscope with Ar, He/Ne, and blue diode lasers.

**Laser microdissection.** Freshly frozen slices of the teratomas 28 days after transplantation were serially cut into 7-μm-thick cryosections. Every seven sections were subjected to immunofluorescence double staining for Ksp-cadherin and Pax-2 as mentioned earlier for detecting renal primordia-like structures. The other sections were mounted on a polyethylene membrane film (PALM Microlaser Technologies, Bernried, Germany) fixed on glass slides. After fixation in methanol for 3 min, specimens were stained with a toluidine blue solution, dehydrated in 100% ethanol, air-dried, and subjected to laser microdissection. The structures corresponding to the Ksp-cadherin
positive ducts/tubules in the serial sections were excised by laser beam and subsequently laser-catapulted into the lid of a 0.5-ml reaction tube coated by mineral oil (Sigma) using the PALM-MB K laser microdissection system (PALM). Total RNA was extracted from the collected cells with an RNA extraction kit (PALM) in accordance with the manufacturer’s protocol and subjected to RT-PCR analysis of the kidney development-related genes as mentioned earlier. Amplification was done with 50 cycles.

RESULTS

RT-PCR analysis of ES outgrowths. In hanging-drop suspension cultures, cells harvested from undifferentiated ES cell cultures (Fig. 1A) developed into EBs (Fig. 1B). Expansion outgrowths in vitro (Fig. 1C) provided cells for RT-PCR and teratoma formation. The EB outgrowths expressed genes essential for kidney development, i.e., Pax-2, Lim-1, c-Ret, and Emx2 in the mesonephric duct and ureteric bud and Sall1, WT-1, Eya-1, GDNF, and Wnt-4 in the metanephric mesenchyme (Fig. 1D). The overall expression pattern was basically comparable among ES cell lines with some variance (Table 3). Pax-2 expression, which regulates the first step in kidney development, became detectable by days 6–9 of expansion, following the expression of other genes.

Fig. 3. Confocal laser scanning microscopy of the ducts in teratoma tissues 14 days after transplantation and of control tissue. All sections were stained for Pax-2 (red) and either endo A cytokeratin, Ksp-cadherin, or DBA (green). A and B: same duct shown in Fig. 2, A–D. Epithelial cells are Pax-2 positive and simultaneously exhibit Ksp-cadherin immunoreactivity (A) or DBA-binding (B) in their membranes. C and D: Pax-2-positive ducts in the transplants originating from ES cell line D3 (C) or R1 (D) cells are simultaneously positive for Ksp-cadherin or endo A cytokeratin. E: some ducts, ~7.5–35 µm along the short axis, are compacted in a small area. The surrounding tissues are negative for Pax-2. F: image of a section at a distance from Fig. 3E. Note that these ducts run in a straight manner and that some ducts have the appearance of blind ends (arrowheads). G: mesonephric nephron-like convoluted tubules. Epithelial cells are positive for Pax-2 and endo A cytokeratin. H–O: control staining of E12 mouse embryos. H and I: in the metanephric region, the ureteric bud branch is positive for both Pax-2 and endo A cytokeratin (H) or Ksp-cadherin (I). Induced mesenchymal cells surrounding the ureteric bud branch are positive for Pax-2 alone. J and K: mesonephric region. Mesonephric tubules are positive for both Pax-2 and endo A cytokeratin (J) or Ksp-cadherin (K). L: urogenital sinus is positive for endo A cytokeratin alone (*). In contrast, the caudal part of the mesonephric duct (arrowhead) is positive for both Pax-2 and endo A cytokeratin. M: ureter is positive for Pax-2 and Ksp-cadherin. N: bile duct is positive for DBA staining, showing the reliability of lectin binding. Endothelial epithelium is negative for Pax-2. O: negative control in the metanephric region. Omitting the step of staining with primary antibody or lectin significantly reduces the staining intensity. Only a few cells in the uninduced mesenchyme show auto fluorescence. Bars = 20 µm.
genes. All gene expression continued up to day 21, the last time point examined.

RT-PCR analysis of ES cell-derived teratomas. To detect developing kidney primordia and their derivatives, transplants of EB outgrowths were analyzed 14 and 28 days after transplantation. Each transplant developed into a teratoma in the host retroperitoneum. RT-PCR analysis detected expression of all the above genes in the transplants at both survival times in each cell line (Fig. 1D, Table 3). Each gene expression, however, cannot be regarded as direct evidence demonstrating the induction of renal primordial cells because it is not specific for kidney development. Thus we subsequently attempted the histochemical detection of renal primordial structures.

Histochemical analyses of ES cell-derived teratomas. Multidifferentiated teratoma tissues were formed in all transplants 14 and 28 days after transplantation, in which the main components were neural tissues, smooth and skeletal muscle tissues, and endodermal ducts/cysts positive for endo A cytokeratin but negative for Pax-2 (Fig. 2, A and B). Immunoreactivity for Pax-2 was observed mainly in the neural cells (not shown). In contrast, renal primordia-like structures were few at both survival times, although they were detectable in most transplants.

Fourteen days after transplantation, a small number of ducts with simple columnar or cuboidal epithelium were positive for both Pax-2 in the nuclei and endo A cytokeratin in the cytoplasm, as well as for DBA lectin binding in the cell membranes (Fig. 2, A–C). This staining pattern is similar to that of mesonephric ducts or ureteric buds (Table 1). No nuclear staining for WT-1 was observed in the tissues adjacent to the ducts (Fig. 2D), indicating that metanephric mesenchyme had not been induced. Branching morphology of the ducts was evident in serial sections, with the ducts branching in a dichotomous manner (Fig. 2, E–H).

Confocal laser-scanning microscopy confirmed that Pax-2-positive epithelial cells of the ducts were simultaneously positive for Ksp-cadherin and DBA binding in their cell membranes (Fig. 3, A–D). In some cases, several ducts varied in

**Fig. 4.** Confocal laser scanning microscopy of renal primordium-like structures in teratoma tissues 28 days after transplantation. All sections were stained for Pax-2 (red) and Ksp-cadherin (green), and with 4′,6-diamidino-2-phenylindole dihydrochloride (blue). A–C: renal primordium-like ducts originating from D3 (A), R1 (B), and 129/sv-ES (C) cells. They are doubly positive for Ksp-cadherin and Pax-2. Note that the duct in A branches in a dichotomous manner (*). Convoluted tubules with Pax-2 immunoreactivity (arrow) are also seen near the ducts (arrowheads) in B. D–G: Pax-2-negative ducts (D and E) and tubules (F and G). They appear to be differentiated judging from the disappearance of Pax-2 immunoreactivity. Mesonephric nephron-like structure (F) has a presumptive rudimental renal corpuscle (arrowhead), in which a small avascular glomerulus can be seen. Bars = 50 μm in A, B, G; 20 μm in C and D; 10 μm in E and F.
diameter were observed in a small area, confirming the branching morphology similar to the ureteric tree (Fig. 3E). Analysis of serial sections revealed that the ducts extended in a straight manner rather than convoluted, and ended blind (Fig. 3F). Cells surrounding the ducts were completely negative for Pax-2, showing that the induced mesenchymal cells were absent around them (Fig. 3, E and F). Convoluted tubules doubly positive for Pax-2 and endo A cytokeratin were also observed (Fig. 3G). Those tubules had no discernible glomerulus and resembled mesonephric tubules rather than metanephric nephrons, because nuclear staining for Pax-2 disappears in the metanephric nephrons when they develop further than the S-shaped body stage (12, 32, 33). Control staining of E12 mouse embryos showed the validity of the histochemistry, in which doubly positive staining for Pax-2 and endo A cytokeratin, Ksp-cadherin, or DBA was confined to the mesonephric duct and nephrons and to the derivatives of the ureteric bud (Fig. 3, H–N). Omitting the primary antibody lectin significantly reduced the staining intensity in those structures of E12 embryos (Fig. 3O).

Twenty-eight days after transplantation, both mesonephric duct-like and mesonephric nephron-like structures were also detectable in the teratoma tissues, but still in small numbers (Fig. 4). Their reactivity for the above antibodies (Fig. 4, A–C) and lectin was basically the same as that of the renal primordia-like structures 14 days after transplantation. In some ducts and tubules, however, Pax-2-immunoreactivity decreased or disappeared (Fig. 4, D–G), indicating their differentiation. Some tubules had a rudimentary renal corpuscle-like portion with an avascular glomerulus (Fig. 4F), which supported that they resembled mesonephric nephrons.

Laser microdissection analysis of renal primordia-like structures. Laser microdissection of Ksp-cadherin-positive ducts/tubules was performed 28 days after transplantation (Fig. 5, A–F), and the extracted RNA samples were subjected to RT-PCR analysis of kidney development-related genes. As a result, expression of Pax-2, Lim-1, c-Ret, Emx2, Sall1, WT-1, Eya-1, GDNF, and Wnt-4 was detected (Fig. 5G), confirming that those structures actually possessed the renal primordia-like features. Expression of HNF3β, an endodermal epithelial marker not involved in kidney development, was undetectable.

DISCUSSION

This study shows that branching ducts with morphological and histochemical features similar to those of mesonephric ducts or ureteric buds were induced in the in vivo outgrowths of the mouse ES cell-derived EBs. They had Ksp-cadherin in the cell-cell junctions, a specific epithelial marker for developing kidney and genitourinary tract (11, 38). Some of the ducts were accompanied by mesonephric nephron-like structures. Notably, expression of kidney development-related genes was detectable in these ducts and mesonephric nephron-like structures. These data suggest that ES cells are capable of differentiating into the duct system of the kidney. The incidence of renal primordial structures in the teratomas was low in all ES cell lines examined. The long-term survival (28 days) did not increase the incidence compared with that in 14-day survival, suggesting that the commitment into the renal cell lineages occurred in EB outgrowths and/or in early stage after transplantation. Judging from the disappearance of epithelial Pax-2-immunoreactivity, the long-term survival appeared to contribute to the differentiation of renal primordial structures. A decrease in Pax-2-immunoreactivity occurs in mesonephric tubules during development (34) and in cortical collecting ducts in the adult kidney (12). Some mesonephric nephron-like structures had a rudimentary glomerulus in contrast to previous studies of human ES cells that reported the presence of nephron-like structures with discernible glomeruli and tubules in ES cell-derived teratomas (1, 43). This discrepancy seems to depend principally on species differences, i.e., the human fetus has a well-developed mesonephros with distinct glomeruli (27), whereas the mouse fetus does not (48). In fact, no
discernible vascularized glomeruli were observed in the mesonephros of mouse embryos used as controls.

Although gene expression essential for kidney development was detected in the EB outgrowths, the pattern of expression did not correlate with that found in mouse knockout experiments (23, 29, 35, 37, 41). For example, expression of GDNF and Wnt-4 preceded Pax-2 expression, the earliest event in kidney development along with the expression of Lim-1. This altered gene expression pattern may be due to the fact that the EB outgrowths contain a wide variety of cell lineages, some of which express the same genes as the developing kidney. Taken together, gene expression in metanephric development is shared with mesonephric development. These facts may be responsible for the absence of discernible metanephric nephron structures in teratomas in contrast to both the in vitro and in vivo PCR data. Thus the present study indicates that simultaneous induction of a duct system and the metanephric mesenchyme might not occur in the EB outgrowths. However, the possibility cannot be excluded that metanephric cells appeared in the EB outgrowths but failed to survive in the teratomas. Metanephric mesenchymal cells, the epithelial progenitor cells of the metanephric nephron, can differentiate into myofibroblasts, smooth muscle, and, likely, endothelial cells in vitro, suggesting that they are pluripotent stem cells of the nephron (26). Further research will be necessary to evaluate the potential of ES cells to differentiate into the metanephric nephron.

The nephron is organized from many cell types, e.g., podocytes, mesangial cells, juxtaglomerular cells, and uriniferous tubule epithelial cells. If these cells are individually induced from stem cells, they may be useful for cell therapy in kidney diseases, or for the development of bioartificial organs in combination with tissue engineering. However, because of the intricate structure of the nephron, employing these differentiated cell types as cellular sources in vitro or in vivo is unlikely to result in the formation of functional structures. Induction of nephrons is crucial to the regeneration of kidney tissues. Given that nephrons develop in conjunction with the collecting duct system, and the connection between them is essential for acquiring the proper function of the nephron, the induction of collecting ducts will be a requirement for this purpose. Previous studies, by both other investigators and ourselves, demonstrated that when renal primordia of experimental animals, including metanephric mesenchyme and ureteric bud, were implanted into adult host kidneys or ectopic sites, they produced a number of functional nephrons with vascularized glomeruli and collecting duct systems (14, 31). Furthermore, metanephric tissue transplanted from rat embryos to adult rats after 3 days of preservation in vitro developed into functional nephrons (30). From these results, one can raise the hypothesis that once renal primordial cells, ureteric bud epithelial cells, and metanephric mesenchymal cells can be produced from ES cells and selectively amplified, coculturing them will induce nephrogenesis accompanied by collecting duct formation via reciprocal inductive interactions. Transplantation of developing nephrons of ES cell origin into an impaired kidney or to ectopic sites is considered to be a possible strategy for regeneration of kidney function in the future. Although more work is necessary to establish in vitro induction and selection (38) techniques for renal primordial cells, the present study demonstrated histochemically the potency of ES cells to differentiate into cells of the mesonephric duct/ureteric bud lineage essential for metanephros development, providing a basis for investigating ES cells as a potential source for kidney regeneration.

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