Development of Endothelial Cell Lines From Embryonic Stem Cells
A Tool for Studying Genetically Manipulated Endothelial Cells In Vitro

Giovanna Balconi, Raffaella Spagnuolo, Elisabetta Dejana

Abstract—Totipotent embryonic stem cells can be induced to differentiate to endothelium in vitro. This may be a useful tool for obtaining cultures of genetically manipulated endothelial cells because embryonic stem cells are relatively easy to transfect and are commonly used for gene inactivation experiments in mice. However, embryonic stem cell–derived endothelial cells could not be easily separated from embryoid bodies and maintained in culture. In this study, we describe the isolation and characterization of immortalized endothelial cell lines obtained from embryonic stem cells differentiated in vitro. The cell lines were analyzed for expression of endothelial cell markers, including growth factor receptors and adhesion molecules, and compared with endothelial cells obtained from the yolk sac, the embryo proper, or the heart microcirculation of the adult. We propose that this approach may be useful for obtaining endothelial cells carrying gene mutations that are lethal at very early stages of development. (Arterioscler Thromb Vasc Biol. 2000;20:1443-1451.)

Key Words: endothelium ■ embryonic stem cells ■ vasculogenesis

The endothelium is the first tissue to differentiate during embryonic development. As early as embryonic day (E) 7.5 of gestation, cells derived from the extraembryonic mesoderm form aggregates that may then evolve into blood islands. The peripheral layers of the blood islands are formed by the differentiating endothelial cells, whereas the cells of the inner part acquire hemopoietic characteristics.1 In the embryo proper, most areas of early mesoderm contain endothelial precursors called angioblasts, which form the embryonic primitive vascular network. The latter will mature through subsequent steps, including sprouting angiogenesis, branching, and remodeling into small and large vessels.2

Embryonic angioblasts express different markers, which are maintained during embryonic vasculogenesis and angiogenesis. These markers are downregulated in adult vessels but can be reexpressed in pathological angiogenesis.1,3,4 The study of the different steps of endothelial cell and vascular differentiation is limited by the difficulties in isolating and culturing endothelial cells from embryos at early stages of development.

Few reports indicate that totipotent embryonic stem (ES) cells can spontaneously differentiate to endothelial cells in vitro.5–8 In the absence of leukemia inhibitory factor (LIF), ES cells lose their pluripotent attributes and differentiate into a monolayer of extraembryonic endoderm- and mesoderm-like cells.9 When cultured in suspension, they form aggregates that resemble mouse blastocysts; hence, they are called embryonic bodies (EBs).10,11 Inside the cells, it is possible to detect vascular-like structures formed by the differentiated endothelial cells.

ES-derived endothelial cells acquire cell-specific markers in a time-dependent manner after LIF removal, reaching maximal differentiation at 11 days. In a previous study, we found that the number of differentiating endothelial cells is increased by culturing ES cells in the presence of several endothelial growth factors, including vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF).12 In addition, it was found that the endothelial markers are expressed in sequential steps, which closely recapitulate endothelial cell differentiation in vivo during embryonic development.12

With this system, however, endothelial cells could not be studied as a purified cell culture because they grow with other cell types. This constitutes an important limitation of the system, which otherwise might have a large series of applications. For instance, targeted inactivation of a few genes may induce a vascular phenotype at early stages of development. Null mutation of VEGF receptor-1 and VEGF receptor-2 prevents complete endothelial cell differentiation13 and normal vascular tubulogenesis, respectively.14 The absence of Tie-2 or angiopoietin-1 expression affects angiogenesis.15–17 These mutations cause lethality within E10.5 and render the culture of endothelial cells from these early embryos difficult (for review, see Reference 18).
In some cases, the mutated genes are not endothelium specific but encode important signaling or adhesive molecules. In these cases, mouse lethality is not due to a vascular phenotype, but the early death of the embryos prevents the study of endothelial functional behavior in culture.

Some studies report the culture of endothelial cells from the yolk sac at E8 to E12. In a recent study, Hatzopoulos et al describe the isolation and in vitro differentiation of endothelial cell precursors starting from E7.5 embryos.

In the present study, we describe a methodology to isolate and culture endothelial cells from ES cells differentiated in vitro. The morphological and functional behavior of these cells was compared with the behavior of endothelial cells obtained from the yolk sac and embryos at E9.5. We found that the ES-derived cell lines present characteristics of differentiated endothelial cells, which in many instances are comparable to those of endothelial cell lines obtained from the yolk sac or the embryo proper. We conclude that the possibility to differentiate and culture the endothelium from ES cells is a useful tool for studying the different steps of endothelial differentiation and for obtaining and studying genetically manipulated endothelial cells.

**Methods**

**Antibodies**

Primary antibodies used in immunofluorescence microscopy, fluorescence activated cell sorting (FACS), and Western blot analyses were (1) rat monoclonal antibodies (mAbs) directed to mouse: vascular endothelial (VE)-cadherin, BV12; platelet endothelial cell adhesion molecule (PECAM)-1, MEC 7.46; functional adhesion molecule (JAM), BV11; zonula occludens-1 (ZO-1) (provided by B. Stevenson, University of Alberta, Edmonton, Canada); MUC18, MEC 12; integrin α,β, and CD34, MEC 14.7 (provided by A. Vecchi, Mario Negri Institute, Milan, Italy); and E-selectin (a gift from D. Vestweber, Institute of Cell Biology ZMBE, Münster).

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**Isolation of Embryonic Endothelial Progenitor Cells**

All tissue culture reagents were purchased from Gibco-BRL (Life Technologies LTD), unless otherwise indicated. Plastics for cell culture were from Falcon (Becton-Dickinson Labware).

Mouse yolk sacs and embryos were separated at E9.5 and were processed as previously described by Fennie et al with minor modifications (Figure 1). After removing the decidua and Reichert’s membrane, embryos and yolk sacs were incubated in collagenase-DNase solution and dissociated by gentle pipetting. The resulting cell suspension was plated in culture wells and infected with PmT-containing vectors. Neomycin-resistant clones were then collected and cultured. p.c. indicates postcoital.

**Figure 1.** Schematic representation of procedure to isolate and culture endothelial cell progenitors from embryos and yolk sacs. After removing decidua and Reichert’s membrane, embryo and yolk sac were incubated in collagenase-DNase solution and dissociated by gentle pipetting. The resulting cell suspension was plated in culture wells and infected with PmT-containing vectors. Neomycin-resistant clones were then collected and cultured. p.c. indicates postcoital.

**Figure 2.** Schematic representation of procedure to isolate and culture endothelial cells from ES cell lines. To induce ES differentiation, LIF and feeder cell layers (STO murine fibroblasts) were removed, and ES cells were cultured in the presence of a cocktail of endothelial cell growth factors (see Methods). Cells formed embryoid bodies, which were then collected and disaggregated in a collagenase-DNase solution by gentle pipetting. Endothelial cell (EC) lines were then developed by (1) direct infection with PmT (cell lines 86/1 and 100/1) and (2) immunomagnetic selection with PECAM mAb–coated magnetic beads followed or not by PmT infection (cell lines, 44BR and 44B, respectively). Insert shows whole-mount preparation of 2 E11 EBs labeled with PECAM mAb providing evidence for vascular-like structures.

Germany; (2) mouse mAbs against α- and β-catenin (Transduction Laboratories); and (3) rabbit polyclonal antibodies to chicken cingulin (a gift of S. Citi, Università di Padova, Padova, Italy); human occludin (Zymed Laboratories, Inc); human N-cadherin (a gift from Dr B. Geiger, Weizmann Institute, Rehovot, Israel); human von Willebrand factor (DAKO A/S); and mouse Flk-1 (Santa Cruz Biotechnology, Inc).

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Expression of Endothelial Differentiation Markers and Growth Factor Receptors in Lines of Endothelial Progenitors

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Expression of membrane-associated markers was evaluated by immunofluorescence microscopy, flow cytometry, and Western blot (see Methods). Intracellular markers were detected by immunofluorescence and, in some instances, Western blot. E-selectin expression was measured after cell activation with 200 U/mL tumor necrosis factor-α (Genzyme Co) for 5 h at 37°C. The expression of growth factor receptors was detected by RNase protection and, for Flt-1 and Flk-1, confirmed by Western blot. 44B and 44BR were derived from CJ7-ES cells; 86/1 and 100/1, from R1 cells; E2 and YS, from the whole embryo or yolk sac microvessels; and H5V, from heart microvasculature. ND indicates not done.

In Vitro Differentiation of Embryonic Endothelial Cell Progenitors

The relevant steps of cell isolation and culture are indicated in Figure 2. CJ7 and R1-ES cells, both 129/Sv-derived, were used. These cell lines were grown in the undifferentiated state either on gelatin (0.1%)–coated Petri dishes (CJ7) or on a feeder layer of STO murine fibroblasts (R1).31

The culture medium of undifferentiated ES cells was DMEM with 15% FBS (Hyclone Laboratories) supplemented with 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 1000 U/mL LIF, 150 μmol/L monothioglycerol (Sigma Chemical Co), 1 mmol/L sodium pyruvate, and 0.1 mmol/L MEM nonessential amino acid.

To isolate ES cell differentiation and EB formation, ES cells were briefly trypsinized and suspended in Iscove’s modified Dulbecco’s medium with 15% FBS (Hyclone Laboratories) supplemented with 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 450 μmol/L monothioglycerol (Figure 2). Vascular differentiation was optimized by the addition of a growth factor cocktail to the culture medium: recombinant human VEGF (Peprotech Inc) was used at 50 ng/mL; recombinant human erythropoietin (Cilag AG), at 2 U/mL; human bFGF (Genzyme), at 100 ng/mL; and murine interleukin 6 (Genzyme), at 10 ng/mL.12

Cells were seeded in bacteriological Petri dishes (1.5 × 10⁶ cells per 35-mm Petri dish) and cultured for 11 days, without further feeding, at 37°C in an incubator with 5% CO₂ in air and 95% relative humidity. The cells formed EBs, and they were routinely examined for the presence of endothelium-like structures by whole-mount preparation,12,32 by using rat mAb MEC 7.46 directly against mouse PECAM as primary antibody and commercial rabbit immunoglobulins to rat immunoglobulin (DAKO) as a secondary antibody.

EBs, submerged in an open dish of glycerol, were photographed by a PM-10AK camera on a SZ-PT dissecting microscope (Olympus Italia SRL). EBs at E11 were collected and disaggregated by 1-hour incubation in 1.5 mg/mL collagenase A (Boehringer-Mannheim) in DMEM plus 5% FBS. After 50 minutes of incubation, 25 μg/mL DNase (Boehringer-Mannheim) was added.

In some cases ES-derived endothelial cells were selected with the use of sheep anti-mouse CD31 mAb–coated magnetic beads (Dynabeads, Dynal AS).33 Briefly, Dynabeads coated with sheep anti-rat IgG were incubated with anti-mouse CD31 mAb3 according to the manufacturer’s instructions. Cells to be selected were detached with a trypsin-EDTA solution from culture vessel and incubated with the conjugated/coated beads. After 30 minutes at 4°C with occasional agitation, the bead-bound cells were recovered, washed 5 times with DMEM plus 10% FBS and once with DMEM without FBS, and then incubated for 5 to 10 minutes at 37°C in a trypsin-EDTA solution to release the beads. The bead-free cells were then centrifuged and resuspended in fresh growth medium for culture. The cells could be immortalized by using PmT-containing vectors.

In some experiments, endothelial cell lines could be obtained without immunoselection. In those cases, after EB disaggregation, the cells were immortalized by PmT (see Figure 2).

Cell immortalization was performed as previously described.34 Briefly, 24 to 48 hours after seeding in 24-well plates (0.5 to 1 × 10⁴ cells per well), the cells were incubated with ~10³ neomycin-resistant colony-forming units of the retrovirus vector N-TKmT in 1 mL of complete medium per well in the presence of 8 μg/mL polybrene (Sigma). The virus-containing medium was replaced 3 hours later with fresh complete medium. Seventy-two hours later, PmT-infected cells were selected by adding the neomycin analogue G418 at 800 μg/mL. Fresh medium containing G418 at 800 μg/mL was replenished 3 times per week. Colonies of G418-resistant cells, usually described below, E2 and YS cell lines were generated from the immortalization of the embryo-derived and yolk sac–derived endothelial cells, respectively.
observed after 20 to 30 days, were detached, pooled, and reseeded in wider wells. The antibiotic selection was maintained for 1 additional month. Confluent cultures were subcultivated, with seeding at $2 \times 10^4$ cells per centimeter squared.

In previous studies, it was observed that PmT specifically immortalizes endothelial cells and not any other cell type. $^{34,35}$ This allows pure endothelial cell lines to be obtained even if starting from a mixed population. $^{36}$ From each crude explant, we obtained $\sim 6$ to 10 endothelial colonies; the other nonendothelial cells tended to be lost within 2 or 3 passages.

In the present study, we used typical cell lines obtained in different ways for further characterization. The 44B line derived from CJ7-ES cells was obtained by immunomagnetic selection; the 44BR line was obtained by immortalization with PmT of 44B; the 86/1 and 100/1 lines were obtained from R1-ES cells by immortalization without previous immunoselection (see Figure 2). The culture medium of ES- and embryo-derived endothelial cells was DMEM with 20% FBS, supplemented with 2 mmol/L glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 50 μg/mL endothelial cell growth supplement (Sigma), and 100 μg/mL heparin (Sigma).

Fluorescence Flow Cytometric Analysis
Fluorescence flow cytometric analysis was performed by a FACStar Plus apparatus (Becton-Dickinson & Co). Cells were detached by trypsin treatment. A cell suspension (200 μL) in PBS supplemented with 2% BSA was incubated with mAbs to be tested for 30 minutes at 4°C. Cells were then washed twice in PBS containing 2% BSA. The second incubation was carried out at 4°C with the use of FITC-conjugated goat anti-rat IgG (50 μg/mL, Jackson Immuno Research Laboratories, Inc). After 30 minutes, the cells were washed 3 times in PBS with 2% BSA and then analyzed.

Western Blot Analysis
Western blot of cell extracts was performed as previously described. $^{36}$

RNase Protection Analysis
RNase protections were conducted with the Ribonuclease Protection Assay System (Pharmingen) according to the manufacturer’s specifications. Products were resolved in 6% denaturing acrylamide gels. Gels were dried and autoradiographed.

Immunofluorescence
Cells to be examined by immunofluorescence were grown to confluence on glass coverslips (13-mm diameter) coated with human fibronectin (7 μg/mL, Sigma). The cells were then fixed with methanol for 3 minutes at $-20^\circ$C or with 3% paraformaldehyde at room temperature for 15 minutes and processed for indirect immunofluorescence microscopy as already described. $^{37}$ For occludin staining, methanol fixation was followed by 2 incubations of 10 minutes, the first with 0.1% saponin (Sigma) in PBS and the second with 0.1% saponin and 0.5% BSA in PBS.

Rabbit anti-rat rhodamine (TRITC-conjugated, Sigma), with or without the presence of fluorescein (FITC-labeled phalloidin, 2 μg/mL, Sigma), swine anti-rabbit rhodamine (TRITC-conjugated), and rabbit anti-mouse rhodamine (TRITC-conjugated, DAKO) were used as secondary antibodies.

After staining, coverslips were mounted in Mowiol (Hoechst, and observations were carried out with a Zeiss Axiopt photomicroscope equipped for epifluorescence. Fluorescence images were recorded on Kodak T-Max 3200 films exposed at 1000 ISO and developed in Kodak T-Max developer for 10 minutes at 20°C.

Capillary Tube Formation by Endothelial Cells Sandwiched Between Collagen Gels or on Matrigel
Three-dimensional cultures of endothelial cells were made as previously described. $^{38,39}$ Briefly, type I collagen (Collaborative Biomedical Product) from rat tail was diluted to a concentration of 1 mg/mL, and the pH was neutralized by adding 1/10 of the volume of 10X MEM. Aliquots of 250 μL were added to each of the 24-well culture plates and incubated at 37°C until gelation occurred. After gel formation, endothelial cells, which were removed from confluent monolayers by trypsin treatment, were plated down onto the gel at a concentration of $1 \times 10^5$ cells per milliliter per well in DMEM supplemented with 20% FBS, 50 μg/mL endothelial cell growth supplement, and 100 μg/mL heparin. After a 24-hour incubation at 37°C, unattached cells were aspirated, and overlaying collagen gels were generated by using the same procedure. Fresh culture medium (1 mL per well) was added after the polymerization of the second collagen layer. The reorganization of endothelial cell monolayer was monitored daily and photographed with a photomicrograph-equipped inverted microscope (Nikon) 9 days after seeding the cells.

For tube formation on Matrigel, $^{30-42}$ aliquots of 250 μL of Matrigel (10 mg/mL, Collaborative Biomedical Product) were dispensed to each culture well in 24-well plates and incubated for 30 minutes at 37°C. Endothelial cells, removed from confluent cultures by trypsin treatment, were seeded in each well at a concentration of $3 \times 10^5$ cells per 100 μL of culture medium. After 30 minutes of incubation at 37°C, 500 μL of medium was added. Capillary tube formation was evaluated by contrast-phase microscopy 3 hours after seeding of the cells. Longer times of incubation did not significantly modify the cell morphological pattern.

In some experiments, 20 ng/mL VEGF (Peprotech Inc) and 3 ng/mL bFGF (Genzyme), alone or in combination, were added at the time of seeding and maintained throughout the experiments.

Results
ES cells were allowed to differentiate to endothelium by removing LIF and culturing the cells in the presence of a mixture of growth factors, as previously described. $^{7,12}$ After 8 to 11 days, ES cells were organized in EBs, and the presence of PECAM-positive cells could be demonstrated by staining the EBs in whole-mount preparations (see Figure 2, insert). The EBs were then disaggregated, and endothelial cell lines were obtained as indicated in Figure 2 and in Methods. Endothelial cells obtained from the embryo were isolated from the yolk sac and the embryo proper and directly immortalized by PmT (see Figure 1).

In the past 3 years, we have performed a total of 28 differentiation experiments of ES cells to endothelium. Of these experiments, 21 were performed with the use of R1 cells, and 7 were performed with the use of CJ7 cells.
The successful experiments, defined as those giving cell lines that could be maintained in culture for >50 population doublings and that expressed endothelium-specific markers, occurred in 13 of the 21 R1 cell experiments and in 3 of the 7 CJ7 cell experiments. The unsuccessful experiments were defined as those in which the cells died within 5 to 7 passages and culture lines could not be established. We could not get any surviving colonies after the first passage in only 2 of the 21 experiments with R1-ES cells.

Greater use of R1 cells was due to the fact that these cells are preferentially used in several laboratories, including ours, for the production of genetically manipulated animals. In addition, the 3 lines obtained from CJ7 did not express a complete set of endothelial markers (see the Table).

In 3 experiments, we did not use PmT. Among the lines that were produced, we studied 44B in detail and compared those cells with cells obtained on the same day and immortalized with PmT (44BR). No major difference in growth curve, life span, or marker expression was observed when 44B and 44BR were compared.

Endothelial cells from ES cells were compared with endothelial cells derived from either the yolk sac or the whole embryo. As shown in Figure 3, the cells grew as monolayers (evaluated by phase-contrast microscopy). Although 44B cells presented a more epithelial-like morphology compared with the other lines, all the lines maintained contact inhibition of cell growth.

ES-derived endothelial cell lines expressed several endothelial markers. Figure 4 shows the FACS analysis of the 44B and 86/1 lines for the expression of the cell adhesion molecules integrin $\alpha_v\beta_3$, PECAM, CD34, and JAM. $^{25,44}$ Whereas 86/1 cells were strongly positive for all the mAbs tested, 44B were positive for only $\alpha_v\beta_3$ and JAM. When the cells were positively stained, they presented a homogeneous pattern, indicating a homogeneous population.

Besides FACS analysis, we studied the distribution of adhesion molecules by immunofluorescence microscopy. Lines 44B and 86/1 (not shown) presented a correct organization of junctions that was comparable to that of E2 endothelial cells (see Figure 5). The endothelium-specific VE-cadherin and the cytoskeletal/signaling catenins ($\alpha$-catenin and $\beta$-catenin) were distributed at intercellular contacts (Figure 5 and not shown). By Western blot, VE-cadherin molecular weight was similar in ES cells and in the other endothelial cell lines examined, indicating that the protein was correctly processed and expressed (not shown).

Other junctional proteins, such as the tight junction components ZO-1, JAM, PECAM, and MUC18 (in the lines for which it was detectable; see Table), were correctly distributed at the intercellular clefts (Figure 5 and not shown).

The Table summarizes the expression pattern of adhesion proteins in the different lines. The 2 tight junction proteins, occludin and cingulin, were absent in all the cell lines (not shown), whereas MUC18, CD34, von Willebrand factor, and PECAM were absent in 44B and 44BR. The integrin $\alpha_v\beta_3$ was found in all cell lines. The pattern of marker expression in embryonic cell lines (86/1, E2, and YS) was comparable to that of cell lines derived from adult heart (H5V) and lung (1G11) microcirculation (Table and not shown). $^{33,34}$

The Table also displays the pattern of the expression of angiogenic markers. Among growth factor receptors, only Tie-2 and Flt-1 were found on 44B cells, whereas 86/1 and 100/1 lines were positive for all the markers examined.

As shown in Figure 6, E2 cells were able to form tubular structures in collagen and Matrigel. Addition of VEGF and bFGF alone or in combination did not significantly modify cord formation (Figure 6, compare panels B and C). ES-
derived endothelial cells (44B, 44BR, and 86/1) formed elongated structures in Matrigel (Figure 6F), and the cells were able to reorganize their monolayer in a cord network within 10 days of culture (Figure 6D and 6E).

Finally, considering the spatial proximity of endothelial progenitors and hemopoietic cells in the YS and in other embryonic regions, we studied whether the EBs or embryo-derived endothelial cells expressed hemopoietic growth factors. By RNase protection assay, all the cells presented macrophage colony–stimulating factor mRNA. In addition, 44B synthesized LIF and granulocyte colony–stimulating factor, whereas the other lines presented stem cell factor mRNA.

Because 44B showed lower expression levels of endothelial markers, we asked whether these cells may have been blocked at an earlier step of differentiation. Therefore, we tested whether promoting endothelial differentiation with 1 mmol/L trans-retinoic acid and 3 mmol/L dibutyryl cAMP (both from Sigma) for 96 hours could induce the expression of the missing endothelial proteins; however, even such treatment did not change the pattern of marker expression (not shown).

**Discussion**

To differentiate in a primary vascular plexus, endothelial cells must acquire several specialized characteristics, such as the...
capacity to respond to vascular growth factors, to form a monolayer, and to get organized into tubular structures. All these responses require the existence of specific cell-to-cell adhesion proteins, receptors for endothelium-specific growth factors such as VEGF, and the reorganization of cytoskeletal proteins. The study of these processes is not an easy task in vivo, and it is not always possible to dissect and understand the reciprocal role of the different variables. The use of genetically manipulated mice and inactivation of a few genes by homologous recombination helped to define some of the crucial factors in the formation, assembly, and maintenance of the vascular system in the embryo (for review, see Reference 18).

Additional information could be obtained by the possibility of isolating and culturing the endothelial cells carrying the mutated gene. In the present study, we describe a methodology for isolating and maintaining in culture endothelial cells derived from ES cells differentiated in vitro. The idea is to obtain ES cells that present the mutation of the gene of interest in both alleles (eg, see Reference 7) and produce endothelial cells lacking the expression of the gene.

The procedures to obtain a pure culture of endothelial cells from ES are relatively simple and reproducible and allow us to generate cell lines. To immortalize the cells, we used PmT, an oncogene that was previously used with success to immortalize endothelial cells from embryos. An endothelial cell line generated in absence of PmT infection was also characterized. For up to 13 passages, these cells do not show significant differences compared with cells that have been immortalized by the oncogene. This suggests that it may be possible to obtain endothelial cell lines with prolonged life spans even in the absence of immortalization.

An important question is how ES-derived endothelial cells are representative of either endothelial progenitors or fully differentiated endothelium. When ES-derived endothelial cells were compared with endothelium cultured from the yolk sac, the embryo proper, or adult heart microcirculation, it appears that the 86/1 line presents essentially the same pattern of differentiation. In contrast, the 44B and 44BR lines lacked a few endothelial cell proteins. The pattern expressed includes early (Flk-1) and late (VE-cadherin) angiogenesis markers, suggesting that the lack of some markers was not due to incomplete differentiation of this cell line. In addition, the 44B line has been selected by using an anti-PECAM mAb, so this protein should have been present at the time of selection and possibly was lost later on in culture.

Even if a systematic comparison of different types of ES cells has not been attempted yet, the fact that lines 86/1 and 44B (and 44BR) derive from 2 different ES lines (R1 and CJ7, respectively) may explain the differences between them. Preliminary data indicate that other R1-derived endothelial cells (line 100/1) present a pattern similar to that of line 86/1.

To study the functional responses of the cells lines, we analyzed the expression of junctional proteins and angiogenesis markers. Junctions are correctly organized, and most of the junctional proteins were expressed in all the ES endothelial cells. Angiogenesis markers were present in 86/1, yolk sac, and embryonic endothelial cells and only partially present in 44B and 44BR cells.

When E2 cells were cultured on collagen or Matrigel, they were able to form cord-like structures. ES-derived endothelial
cells in Matrigel formed cordlike structures, and they were able to spontaneously reorganize in cords when maintained in culture for a few days. Overall, these data indicate that ES-derived endothelial cells have the capacity to form cordlike structures in suitable culture conditions. All the cell lines expressed macrophage colony–stimulating factor and other hematopoietic growth factors. This may have functional implications considering that in blood islands, in some regions of the embryo, and in EBs, hematopoietic cells differentiate in close contact with endothelial cells, which may therefore act as inducers of hematopoietic differentiation.

In conclusion, ES cells may be a source of endothelial cells after differentiation in vitro. These cells present characteristics comparable to the endothelium of embryonic vessels and the adult microcirculation. In contrast to endothelial cells, ES cells are relatively easy to transfect. This methodology would allow us to obtain endothelium-carrying gene mutations or the overexpression of desired genes without the eventual development of the mutant mice.

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References


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