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CHARACTERIZATION OF NOVEL CLONAL MURINE ENDOTHELIAL CELL LINES WITH AN EXTENDED LIFE SPAN

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SUMMARY

A murine endothelial cell line was recently established from microvessels that had invaded a subcutaneous sponge implant (Dong, Q. G.; Bernasconi, S.; Lostaglio, S., et al. Arterioscl. Thromb. Vasc. Biol. 17:1599–1604; 1997). From these sponge-induced endothelial (SIE) cells, we have isolated two subpopulations endowed with different phenotypic properties. Clone SIE-F consists of large, highly spread cells that have a relatively slow growth rate, form contact-inhibited monolayers, do not grow under anchorage-independent conditions, express elevated levels of thrombospondin-1 (TSP-1) and are not tumorigenic in vivo. In contrast, clone SIE-S2 consists of small, spindle-shaped cells that have a high proliferation rate, do not show contact-inhibition, grow under anchorage-independent conditions, express very low levels of TSP-1 and are tumorigenic in vivo. Both clones express the endothelial markers vascular endothelial-cadherin and vascular intercellular adhesion molecule-1, but do not express CD31 and E-selectin. In addition, SIE-S2 cells, but not SIE-F cells, express the α-smooth muscle actin isoform. SIE-S2 cells, but not SIE-F cells, are able to form branching tubes in fibrin gels. The SIE-F and SIE-S2 clones, which have properties of nontransformed and transformed cells, respectively, should provide useful tools to investigate physiological and pathological processes involving vascular endothelium.

Key words: endothelium; mouse; angiogenesis; thrombospondin.

INTRODUCTION

Vascular endothelial cells (ECs) play a key role in several physiological or pathological processes such as angiogenesis, control of vascular tone and permeability, wound healing, inflammation, atherosclerosis, and tumor growth and metastasis (Cines et al., 1998). Much of our understanding of the role of ECs in these events has been made possible by the development of techniques for the isolation and culture of ECs from different types of vessels. However, primary cultures of ECs have a number of disadvantages, which include a relatively short life span, the frequent requirement for exogenous growth factors, the possibility of fibroblast and/or smooth muscle cell contamination, and the heterogeneity of ECs obtained from different isolates, resulting in batch-to-batch variability in functional assays.

To circumvent the limitations associated with primary EC cultures, many laboratories have established immortalized EC lines. Transfection or infection with viral oncogenes has allowed generation of EC lines endowed with an extended life span, but which have frequently lost endothelial-specific differentiation properties and thus differ considerably from their normal counterparts (Williams et al., 1988; O'Connell and Edidin, 1990; Durieu-Trautmann et al., 1991; Harder et al., 1991; Ades et al., 1992; Fickling et al., 1992; Vicart et al., 1993; Roux et al., 1994; Fontijn et al., 1995; Lechardeur et al., 1995; Candal et al., 1996; Kanda et al., 1996; Moldovan et al., 1996; Schweitzer et al., 1997). On the other hand, only a few spontaneously immortalized EC lines are available (Gumkowski et al., 1987; Cockerill et al., 1994; Bastaki et al., 1997).

A number of important physiopathological events in which ECs are involved (e.g., angiogenesis and inflammation) occur at the level of the microvasculature rather than in large vessels. It is now clearly established that ECs derived from the microvasculature exhibit distinct phenotypic and functional characteristics that differ significantly from those of large vessel ECs (Gumkowski et al., 1987; Belloni and Tressler, 1990). Unfortunately, the isolation and culture of microvascular ECs is difficult and time-consuming. Recently, a murine endothelial cell line was established from microvessels that had invaded a subcutaneous sponge implant (Dong et al., 1997). These sponge-induced endothelial (SIE) cells were reported to express several endothelial cell markers and to lack tumorigenic potential when injected in nude mice (Dong et al., 1997; A. Vecchi, pers. comm.). Here, we report the subcloning of SIE cells, and the resulting isolation of two distinct populations, one of which exhibits properties of nontransformed and the other of transformed endothelial cells.

MATERIALS AND METHODS

Cells. Parental SIE cells were kindly provided by Dr. A. Vecchi (Milan, Italy) and cultured as described by Dong et al. (1997). SIE clones were obtained by limiting dilution and were subcultured in gelatin-coated flasks

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in Dulbecco's minimal essential medium (Life Technologies, Basel, Switzerland) supplemented with either 10% fetal calf serum (HyClone Laboratories, Logan, UT) or 10% donor calf serum (Life Technologies), 1 m*M* sodium pyruvate, 1% nonessential amino acids, penicillin (100 U/ml), and streptomycin (110 g/ml).

Antibodies. Polyclonal antibodies against mouse vascular endothelial (VE)-cadherin and the anti-mouse CD31 monoclonal antibody MEC 13.3 were kindly provided by M. Corada and E. Dejana (Milan, Italy). Rat hybridoma cells secreting the anti-mouse vascular intercellular adhesion molecule-1 (VCAM-1) monoclonal antibody M/K-1.9 were from the American Type Culture Collection (Manassas, VA). Rat anti-mouse E-selectin monoclonal antibody 10E9.6 was kindly provided by D. Vestweber (Munster, Germany). Monoclonal antibodies (clone 14A) that specifically recognize the alpha-smooth muscle (α-SM) actin isoform in a variety of species, including mouse, were purchased from Sigma Chemical Co. (St. Louis, MO). Monoclonal antibodies to mouse thrombospondin-1 (TSP-1) were purchased from Transduction Laboratories (Lexington, KY).

Immunocytochemistry. Subconfluent cultures of SIE-F and SIE-S2 cells on gelatin-coated coverslips were fixed and permeabilized as previously described (Cavallaro et al., 1998). The cultures were then incubated with primary antibodies for 30 min at 37° C, followed by tetramethylrhodamine isothiocyanate-labeled secondary antibodies (Dakopatts, Glostrup, Denmark), and were routinely counterstained with fluorescein isothiocyanate (FITC)-labeled phalloidin (Sigma) to visualize F-actin. For fluorescence-activated cell sorting (FACS) analysis, SIE clones were either left untreated or treated with 100 U/ml interleukin-1 (IL-1) for 4 h. Cells were then harvested and processed as previously described (Chirivi et al., 1993).

Immunoblotting analysis. Subconfluent cultures of SIE-F and SIE-S2 cells were washed and lysed in ice-cold lysis buffer, containing 50 m*M* Tris–HCl (pH 8), 150 m*M* NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 1 m*M* Na_3VO_4, 10 m*M* NaF, 1 m*M* phenylmethylsulfonyl fluoride, and protease inhibitors from Complete kit (Boehringer Mannheim). Cell lysates were centrifuged to remove cell debris, resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose at 150 mA for 16 h. Blots were then probed with specific antibodies, followed by peroxidase-conjugated secondary antibodies (Pierce, Rockford, IL). The SuperSignal chemiluminescence kit (Pierce) was used to detect immunoreactive proteins.

Assays of capillary tube morphogenesis. To evaluate the ability of SIE-F and SIE-S2 clones to form tubular structures in vitro, cells were grown under each of the following conditions: (1) sandwiched between two collagen layers (Montesano et al., 1983); (2) grown to confluence on the surface of a collagen gel and subsequently incubated with basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), or both agents together, as previously described (Montesano et al., 1986; Pepper et al., 1992) or (3) suspended within a three-dimensional fibrin gel (Montesano et al., 1990). For preparation of fibrin gels, bovine fibrinogen (Sigma) was dissolved in calcium-free MEM (Life Technologies) to obtain a final protein concentration of 2.5 mg/ml. Cells were suspended in the fibrinogen solution at a concentration of 1×10^4 cells/ml, and clotting was initiated by adding 1:10 v/v $CaCl_2$ (2 mg/ml) and thrombin (Sigma, 25 U/ml). The mixture was immediately transferred into either 35-mm dishes or 16-mm wells and allowed to gel for at least 2 min at room temperature before adding complete culture medium. Trasylol (Bayer Pharma, Zurich, Switzerland) was added to the culture medium at a concentration of 200 KIU/ml to prevent lysis of the fibrin substrate (Montesano et al., 1987). Collagen and fibrin gel cultures were processed for electron microscopy as previously described (Montesano et al., 1991).

Assays of anchorage-dependent and -independent growth. To assay for proliferation rate under anchorage-dependent conditions, SIE-F and SIE-S2 cells were seeded into 22-mm gelatin-coated tissue culture wells at a concentration of 2×10^4 cells/well. After 1, 2, 3, and 4 d, media were changed and cells in triplicate wells in each of three separate experiments were trypsinized and counted with a hemocytometer. To assay for anchorage-independent growth, 1×10^4 cells/ml were suspended in agarose gels as previously described (Montesano et al., 1991) and cultured at 37° C with medium renewal every 2–3 d. The cultures were fixed after 14 d with 2.5% glutaraldehyde in 100 m*M* sodium cacodylate buffer (pH 7.4) and examined to assess colony formation.

Tumorigenesis assays. Female NCr-nu/nu mice, 6–8 wk old, were obtained from NCI, DTP, Frederick, MD. SIE clones were injected subcutaneously into the flank of the mice, and tumor growth was monitored twice weekly. Tumor weight was calculated as previously described (Albini et al., 1992). Procedures involving animals and their care conformed with institutional guidelines, that are in agreement with national (D.L. n. 116, G.U., Suppl. 40; Circolare n. 8, G.U.) and international laws and policies (European Economic Community Council Directive 86/609, OJ L 358.1; Guide for the Care and use of Laboratory Animals, United States National Council, 1996).

Results

When seeded at low density, parental SIE cells appeared to be morphologically heterogeneous, consisting of two distinct cell populations: a majority of small, spindle-shaped cells with refractile cell borders, and a minority of large, irregularly shaped, highly spread cells (Fig. 1*A*). To determine whether the two types of endothelial cells might be endowed with different functional properties, we set out to clone parental SIE cells. Out of six clones obtained by limiting dilution, five clones (SIE-S1 to SIE-S5) were composed of small, spindle-shaped cells (Fig. 1*B*) and one clone (SIE-F) by large, flattened cells (Fig. 1*C*). The SIE-S2 clone (Fig. 1*B*) was further characterized as a prototype of all SIE-S clones.

The proliferation rate of the SIE-S2 clone was much higher than that of SIE-F cells (Fig. 2). In addition, SIE-S2 cells were not contact-inhibited and formed multilayers in postconfluent cultures, while SIE-F cells formed a regular, contact-inhibited monolayer, even when maintained at postconfluence for several days (Fig. 3). These findings suggested that SIE-S2 and SIE-F cells had a transformed and nontransformed phenotype, respectively. To investigate this possibility further, we compared the ability of the two clones to grow under anchorage-independent conditions, a parameter widely used to assess cell transformation (Guan and Shalloway, 1992; van Dam et al., 1998). We found that SIE-S2 cells formed numerous colonies in agarose gels, while SIE-F cells remained as apparently single cells and did not form colonies (Fig. 4). Despite their nontransformed phenotype, SIE-F cells have been passaged more than 70 times over a 15-mo period without appreciable loss of proliferative capacity. Interestingly, SIE-S2 and SIE-F cells proliferate in serum-free medium supplemented with insulin and bFGF (data not shown).

To verify that during cell cloning we had not selected contaminating nonendothelial cells, all SIE clones were subjected to phenotypic characterization by immunocytochemical and biochemical techniques using antibodies specific for endothelial and smooth muscle cells. By immunofluorescence staining, VE-cadherin (Lampugnani et al., 1992; Navarro et al., 1995; Breier et al., 1996) was detected at the cell surface of both SIE-F and SIE-S2 clones, confirming the findings obtained with parental SIE cells (Dong et al., 1997). However, the endothelial cell marker CD31 (Newman, 1997), reported to be present in parental SIE cells (Dong et al., 1997), was not expressed by SIE-F or SIE-S2 cells, as assessed by Northern blot, immunoblotting, and FACS analyses. To confirm their endo-

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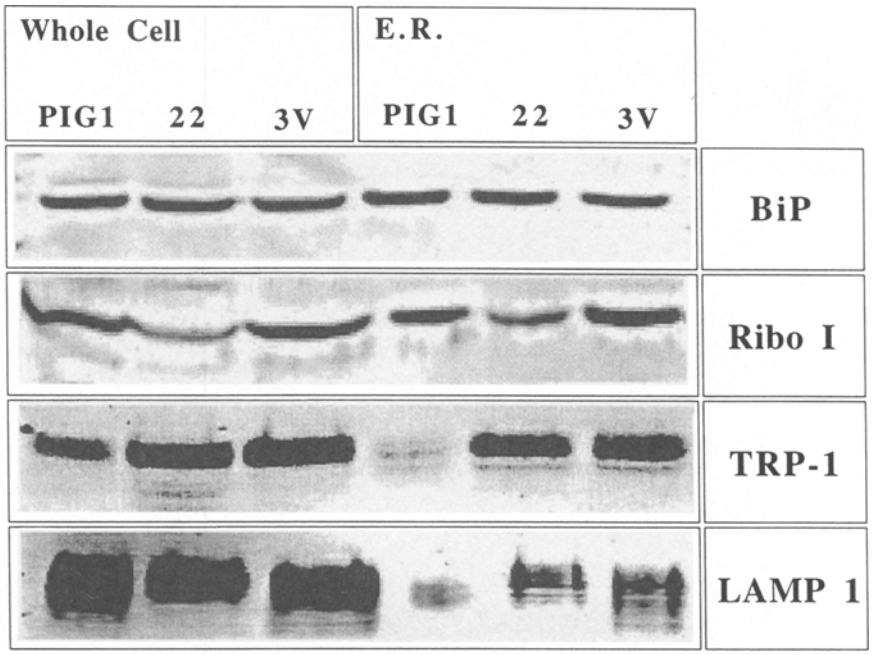
FIG. 1. Morphology of SIE clones. (*A*) Parental SIE cells consist of two morphologically distinct subpopulations: a minority of large, highly spread cells (*arrows*), and a majority of smaller, spindle-shaped cells. (*B*) Clone SIE-S2 is composed exclusively of spindle-shaped cells. (*C*) Clone SIE-F is composed exclusively of large, flattened cells. *Bar*, 100 μm.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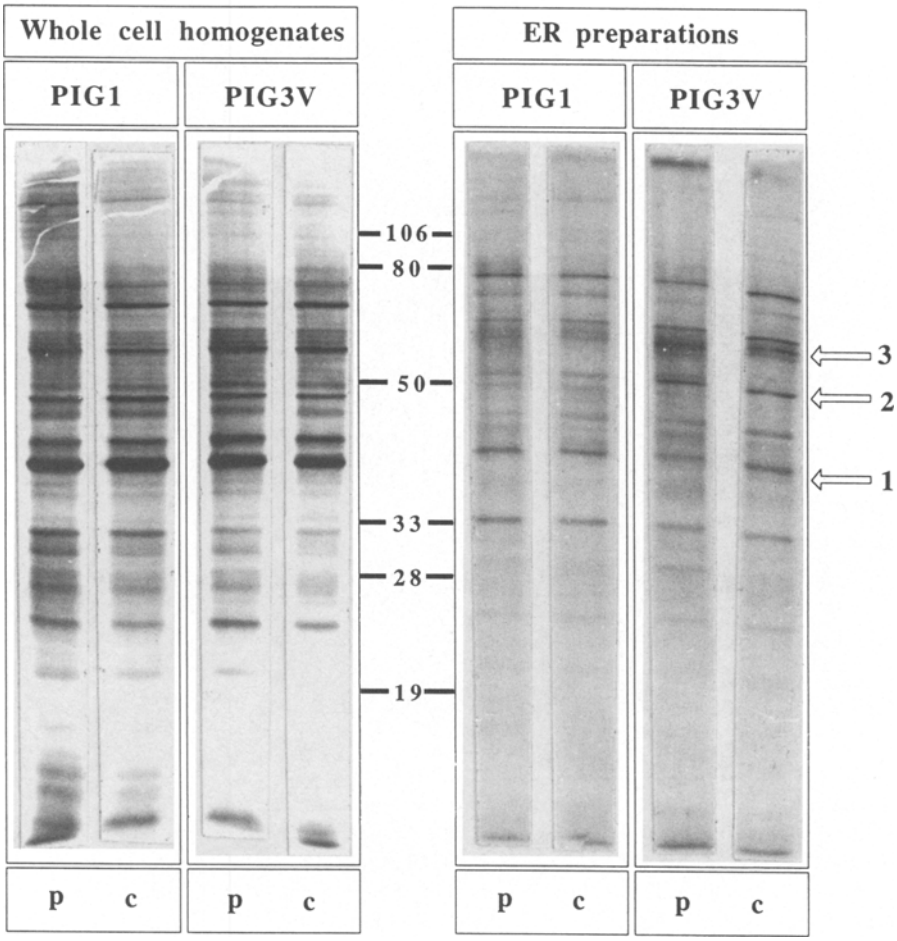
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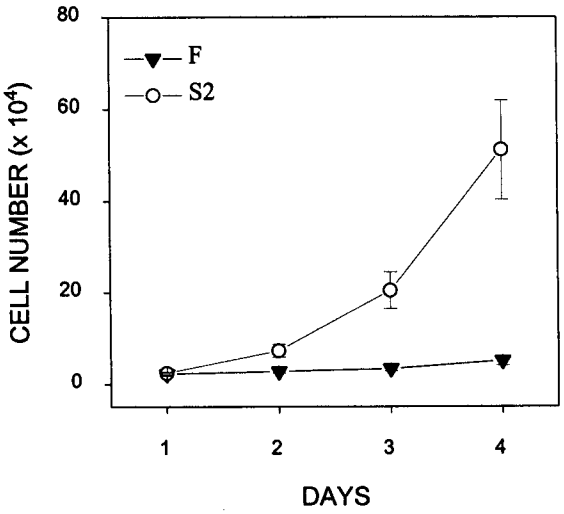

FIG. 2. Proliferation rate of SIE clones. SIE-S2 and SIE-F cells were seeded at 2 × 10^4 cells/well in 12-well plates and counted after 1, 2, 3, and 4 d as described in Materials and Methods. Results are expressed as mean values ± SEM from triplicate determinations in each of three separate experiments.

thelial phenotype further, SIE clones were assayed for the expression of VCAM-1. By FACS analysis, 33% of SIE-S2 cells showed expression of cell surface VCAM-1, which was slightly upregulated (44% of cells) by IL-1 treatment. In contrast, untreated SIE-F cells expressed no VCAM-1, whereas 98% of cells became positive upon IL-1 treatment (data not shown). Expression of the endothelial cell marker E-selectin could not be detected in either the SIE-F or SIE-S2 clone, both in unstimulated and IL-1–treated cells. As expected, the SIE-F clone showed no reactivity with a monoclonal antibody against α-SM actin (Fig. 5). Surprisingly, however, we observed that about 90% of SIE-S2 cells expressed α-SM actin in a pattern colocalizing with stress fibers (Fig. 5). These observations were confirmed by immunoblotting analyses of cell lysates from SIE clones using the anti–α-SM actin antibody. The 42-kDa band corresponding to α-SM actin was clearly detected in lysates from the SIE-S2 clone, while no signal could be observed in lysates from SIE-F cells (Fig. 6*A*). It is noteworthy that α-SM actin was also expressed by the other SIE-S clones (not shown). The results of the phenotypic characterization of SIE clones are summarized in Table 1.

To evaluate the ability of SIE clones to generate three-dimensional tube-like structures, we used three different in vitro assays of capillary morphogenesis. In the collagen gel sandwich assay (Montesano et al., 1983), neither SIE-F nor SIE-S2 cells were able

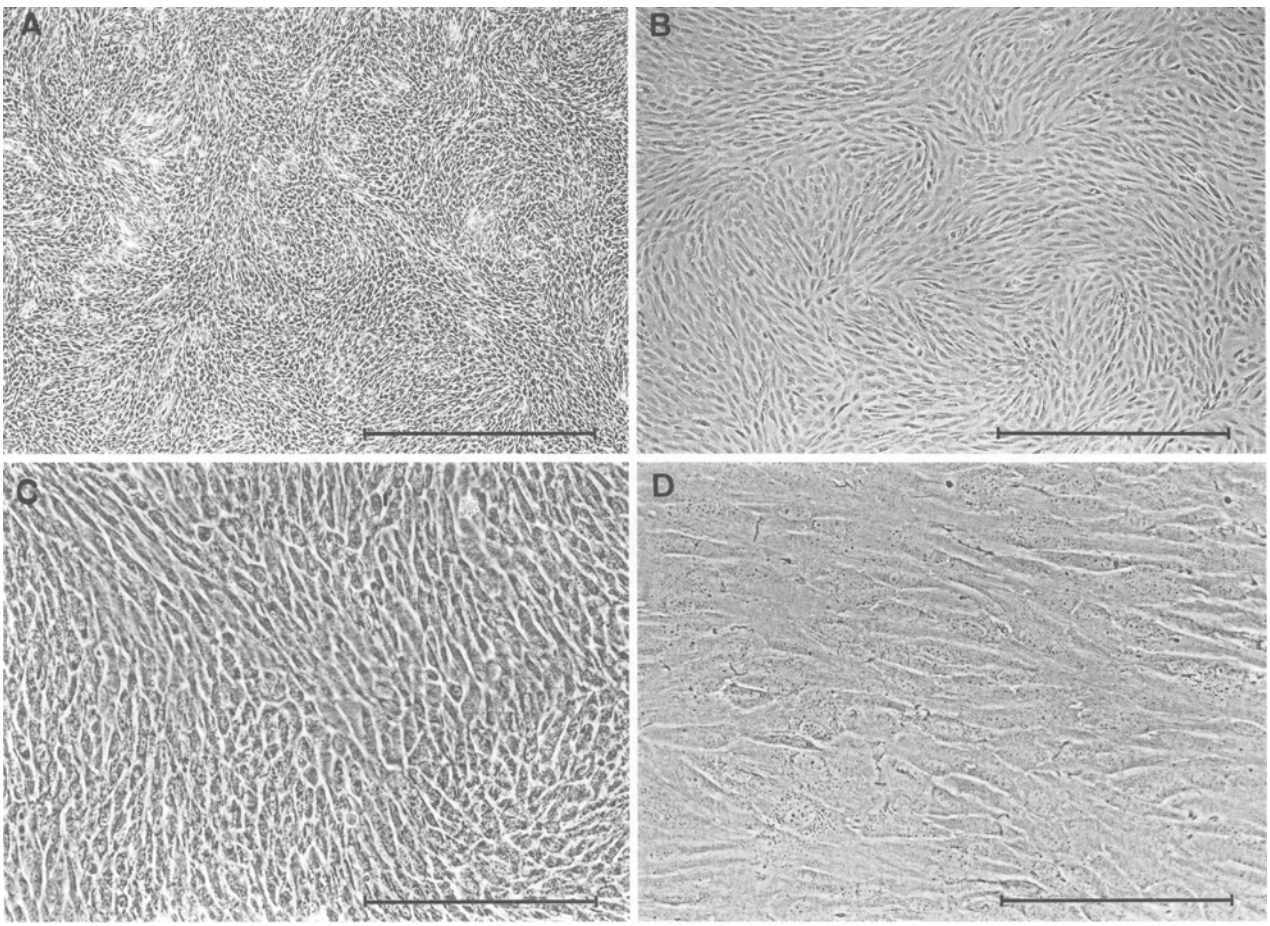

FIG. 3. Postconfluent cultures of SIE clones. SIE-S2 and SIE-F cells were seeded into 35-mm dishes at 4 × 10^4 cells/dish and grown for 5 d. (*A,C*) SIE-S2 cells reach an elevated saturation density, and regions of cell stratification can be observed. (*B,D*) SIE-F cells form a contact-inhibited monolayer. (*A,B*) *Bar*, 1 mm; (*C,D*) *Bar*, 200 μm.

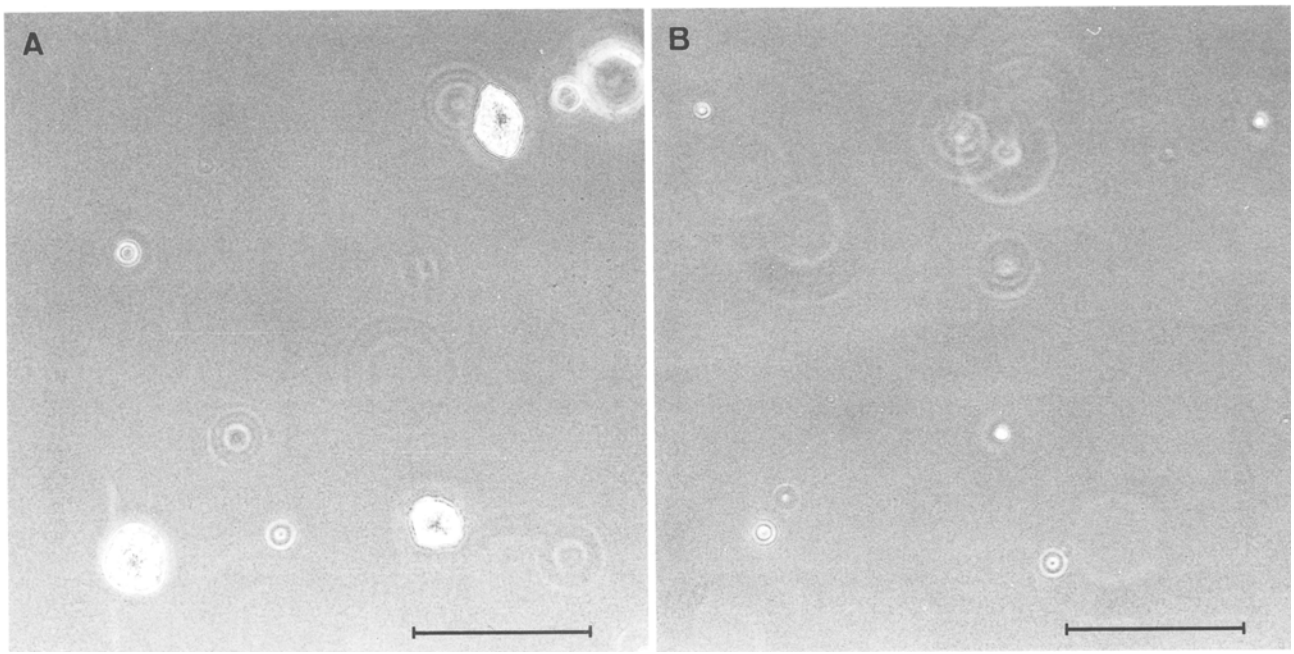

FIG. 4. Assay of anchorage-independent growth. SIE-S2 (*A*) and SIE-F (*B*) cells were suspended in agarose gels as described in Materials and Methods. After 14 d, the cultures were fixed and photographed. SIE-S2 cells form distinct multicellular colonies, whereas SIE-F cells remain as apparently single cells. *Bar*, 200 μm.

to form capillary-like structures (data not shown). In the collagen gel invasion assay, in which bovine microvascular endothelial cells have been shown to penetrate the underlying matrix and to form tubular structures in response to either bFGF or VEGF (Montesano et al., 1986; Pepper et al., 1992), SIE-F cells, and to a greater extent SIE-S2 cells, spontaneously invaded the collagen gel, either as single cells or as thin cords. Incubation with bFGF slightly increased cell invasion, but did not result in the formation of lumen-containing tubular structures (data not shown). However, in another in vitro model of capillary morphogenesis, in which endothelial cells are suspended in a three-dimensional fibrin matrix (Montesano et al., 1990), SIE-S2 cells formed long, extensively branched cords (Fig. 7*A* and *B*). Electron microscopic examination of the fibrin gel cultures showed that some of the cords contained a well-defined central lumen (Fig. 7*C*). Semithin sections of SIE-F cells grown under the same experimental conditions revealed the presence of very thin cords devoid of a central lumen (data not shown). Lack of tube formation was also observed following suspension of SIE-F cells in collagen gels or collagen/Matrigel mixtures, either under control conditions or in the presence of basic FGF, VEGF, or a combination of both agents.

Cultured endothelial cells have been shown to constitutively synthesize high levels of TSP-1, an extracellular matrix protein with purported anti-angiogenic properties, while transformed endothelial cells have been shown to produce little or no TSP-1 (RayChaudhury et al., 1994). In light of these findings, we examined the expression of TSP-1 in SIE-F and SIE-S2 cells. By immunoblotting analysis, we detected high levels of TSP-1 in lysates from the SIE-F clone, whereas TSP-1 was barely detectable in SIE-S2 cells (Fig. 6*B*). These data lend further support to the notion that SIE-S2 cells exhibit a transformed phenotype.

To assess whether the transformed phenotype of SIE-S2 cells might correlate with in vivo tumorigenicity, cells were injected subcutaneously into nude mice. SIE-S2 cells were highly tumorigenic, since all injected mice developed tumors within a few days after cell inoculation (Fig. 8). In contrast, no tumors developed in mice inoculated with SIE-F cells, even at 60 d postinjection. SIE-F cells also failed to form tumors when injected as a suspension in Matrigel, a reconstituted extracellular matrix that promotes the growth of some tumor cell types in vivo (Fridman et al., 1991; Albini et al., 1992) (data not shown). It can therefore be concluded that the SIE-S2 clone has a transformed phenotype in vitro as well as high tumorigenic potential in vivo, while SIE-F cells do not exhibit properties of transformed or tumorigenic cells.

DISCUSSION

We have isolated and characterized two distinct clonal subpopulations derived from the SIE murine endothelial cell line (Dong et al., 1997). One subpopulation (clone SIE-F) exhibits properties of contact-inhibited, nontumorigenic endothelial cells, whereas the other subpopulation (clone SIE-S2) consists of endothelial cells that are morphologically transformed in vitro and tumorigenic in vivo. Both SIE-F and SIE-S2 cells retain several EC-specific properties that were also detected in the parental SIE cell population (Dong et al., 1997), such as the expression of VE-cadherin and VCAM-1. However, when compared to parental SIE cells (Dong et al., 1997), both SIE-F and SIE-S2 cells appear to have lost expression of the endothelial cell marker CD31. Since loss of CD31 expression was previously observed in ECs isolated from mouse aorta, brain, and heart (Bastaki et al., 1997), it is possible that mouse ECs have a propensity to downregulate CD31 during extended passage in vitro.

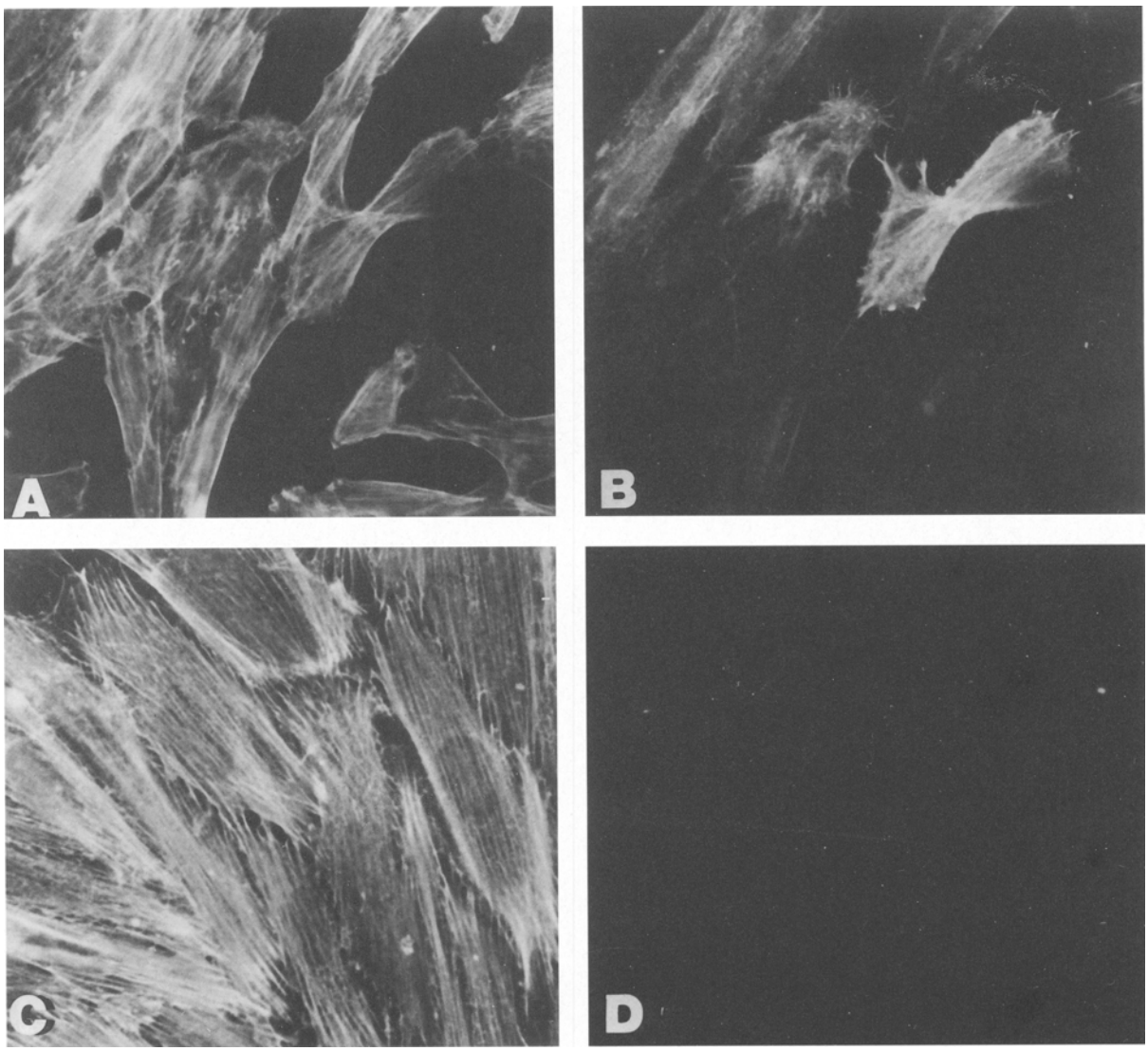

FIG. 5. Differential expression of α-SM actin in SIE clones. SIE-S2 (*B*) and SIE-F (*D*) cells were subjected to immunofluorescent staining with an anti–α-SM actin monoclonal antibody, as described in Materials and Methods. (*A,C*) Cells were counterstained with FITC-conjugated phalloidin to visualize F-actin.

The finding that SIE-S2 cells (and all other SIE-S clones) express α-SM actin was unexpected, since the presence of this actin isoform in ECs has been reported only under specific circumstances. Thus, in the developing avian heart, α-SM actin is expressed in ECs undergoing transforming growth factor (TGF)-β3–induced conversion to mesenchymal cells (Nakajima et al., 1997). In vitro, TGF-β1 induces α-SM actin expression in bovine aortic ECs, concomitantly with the downregulation of endothelial cell markers (Arciniegas et al., 1992). Thus, while TGF-β1–treated bovine aortic ECs undergo a phenotypic transition to smooth muscle-like cells, SIE-S clones retain phenotypic features of both endothelial and smooth muscle cells. Interestingly, co-expression of endothelial and smooth muscle cell markers was previously observed in poorly differentiated vascular cells (TTB cells) isolated from Kaposi's sarcoma-like lesions of HIV-1 *tat* transgenic mice (Cavallaro et al., 1996). Like SIE cells, TTB cells were assumed to have been derived from blood vessels involved in an angiogenic process. It is therefore conceivable that EC activation or recruitment results in dedifferentiation toward a mixed endothelial/smooth muscle cell phenotype. In this respect, it is noteworthy that subcloning of TTB cells also yielded one endothelial-like clone, while all other clones expressed both endothelial and smooth muscle cell markers (U. Cavallaro, unpublished). An alternative possibility is that expression of α-SM actin in SIE-S2 cells reflects a dedifferentiation event associated with cell transformation (*see* below).

The growth properties of SIE-F and SIE-S2 cells were markedly different. SIE-F cells proliferate slowly, form contact-inhibited monolayers and do not form colonies in agarose, which suggests that this clonal population is comprised of nontransformed endothelial cells. This conclusion is further supported by the finding that their inoculation in nude mice did not result in tumor formation. Despite their nontransformed phenotype, SIE-F cells have been passaged more than 70 times over a 15-mo period, without appreciable loss of proliferative capacity. Therefore, although it cannot yet be claimed that they are truly immortalized, SIE-F cells

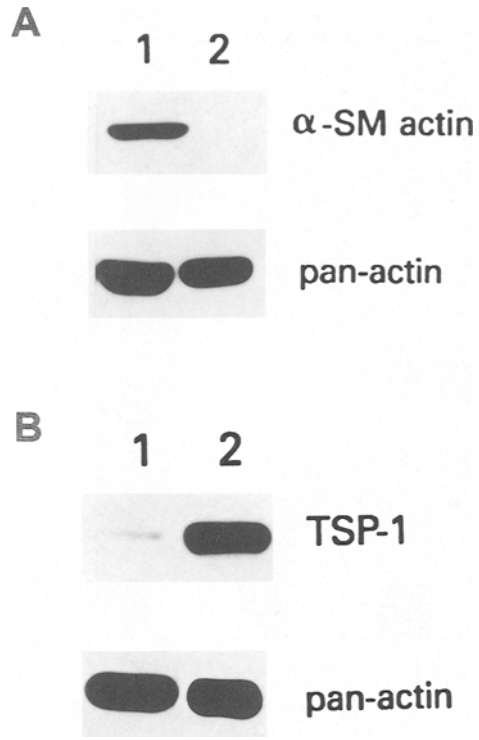

FIG. 6. Western blot analysis for α-SM actin and TSP-1. (*A*) 50 mg of total protein from SIE-S2 (*lane 1*) or SIE-F (*lane 2*) cell lysates were resolved by SDS-PAGE and immunoblotted with a monoclonal antibody against α-SM actin (*upper panel*). Equal loading was confirmed by immunoblotting with polyclonal IgG recognizing all actin isoforms (pan-actin; *lower panel*). (*B*) 50 μg of total protein from SIE-S2 (*lane 1*) or SIE-F (*lane 2*) cell lysates were resolved by SDS-PAGE and immunoblotted with an anti–TSP-1 monoclonal antibody (*upper panel*). Equal loading was confirmed by anti–pan-actin immunoblotting (*lower panel*).

TABLE 1

PHENOTYPIC CHARACTERIZATION OF SIE CLONES[a]

	SIE-F	SIE-S2
VE-cadherin[b]	+	+
CD31[b,c,d]	−	−
VCAM-1[d]	+	+
E-selectin[d]	−	−
α-SM actin[b,c]	−	+

[a] SIE-F and SIE-S2 cells were subjected to immunocytochemical or biochemical analysis as described in Materials and Methods to assess the expression of the markers indicated.
[b] By immunofluorescence staining.
[c] By Northern blotting.
[d] By FACS analysis.

represent, in practical terms, a cell line with an extended life span.

Unlike SIE-F cells, SIE-S2 cells proliferate rapidly, are not contact-inhibited, and grow under anchorage-independent conditions. These findings, coupled with their tumorigenic potential in vivo, suggest that SIE-S2 cells (and the other SIE-S clones) are transformed endothelial cells. Since parental SIE cells (Dong et al., 1997) were not tumorigenic (A. Vecchi, pers. comm.), it is conceiv-

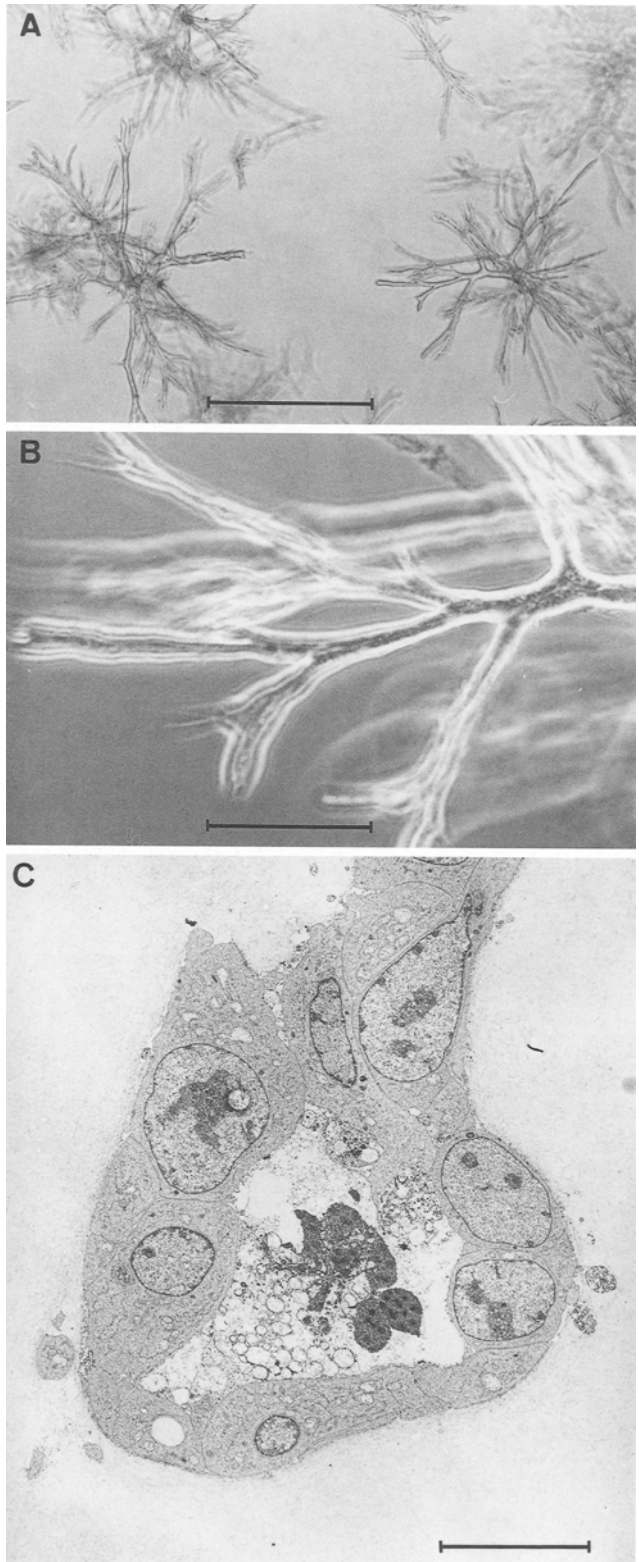

FIG. 7. Tubular morphogenesis of SIE-S2 cells in fibrin gels. SIE-S2 cells were suspended in fibrin gels as described in Materials and Methods and grown for 4 d. (*A*) The cells form extensively branched cords. *Bar*, 500 μm. (*B*) Higher magnification of a branching cord from the colony shown in the *right portion* of *panel A*. *Bar*, 100 μm. (*C*) Thin section of a cord showing the presence of a central lumen containing cell debris. *Bar*, 10 μm.

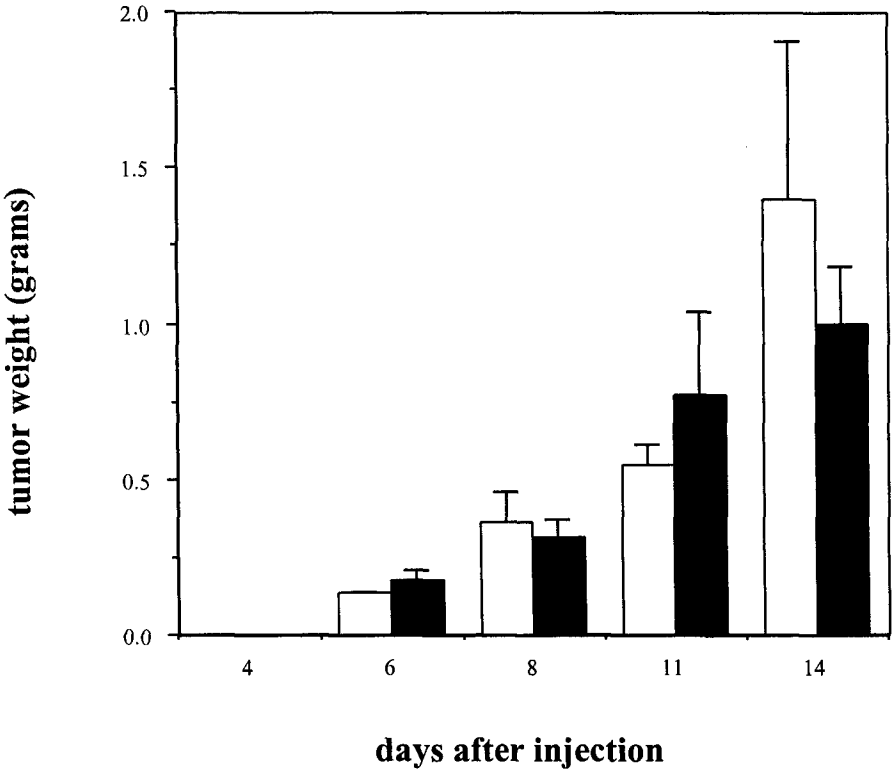

FIG. 8. Tumorigenicity of SIE-S2 cells: 2 × 10^6 (*white bars*) or 5 × 10^6 (*black bars*). SIE-S2 cells were injected subcutaneously into four nude mice/group as described in Materials and Methods. Tumor weights were measured in each group of mice (data represent the mean ± SD).

able that a transformation event occurred during the initial passaging of these cells, resulting in a mixed population of nontransformed (SIE-F) and transformed (SIE-S) cells, which we have now isolated and propagated in homogeneous culture.

TSP-1 is an extracellular matrix protein with anti-angiogenic activity that is abundantly expressed by normal ECs but is downregulated in transformed ECs (RayChaudhury et al., 1994). Induction of TSP-1 expression restored a normal phenotype in transformed ECs (Sheibani and Frazier, 1995). Our finding that SIE-F cells express high levels of TSP-1, whereas SIE-S2 cells do not, corroborates the conclusion that SIE-S2 cells have a transformed phenotype. Whether downregulation of TSP-1 in SIE-S2 is a cause or consequence of transformation is not known.

When grown under conditions which we have found promote the formation of capillary-like tubes by human umbilical vein endothelial cells, bovine micro- and macrovascular endothelial cells, and transformed murine endothelial cells (Montesano et al., 1983, 1986, 1990; Pepper et al., 1992), SIE-F cells were unable to organize into tubular structures. This indicates that despite their nontransformed phenotype, SIE-F cells do not express morphogenetic properties in vitro. In this respect, it is noteworthy that other immortalized endothelial cell lines, such as HMEC-1 cells (Ades et al., 1992) and RBE-4 (Roux et al., 1994), were also found to lack morphogenic potential in vitro (R. Montesano and M. S. Pepper, unpublished). In contrast to SIE-F cells, SIE-S2 cells were able to form extensively branched cords when suspended in three-dimensional fibrin gels. The finding that some of these cords contain a well-defined central lumen suggests that, despite their transformed phenotype, SIE-S2 cells have at least partially maintained the capacity to organize into tubular structures resembling capillary blood vessels. We have no explanation at present as to why SIE-S2 cells form tubes in fibrin gels but not in collagen gels. It can be speculated, however, that SIE-S2 cells express a specific repertoire of integrins that allows them to polarize (and therefore to form a lumen) in a fibrin matrix but not in a Type-I collagen matrix.

Like most immortalized cell lines currently available, SIE-F and SIE-S2 cells express only some of the differentiated functions of ECs and may therefore have limitations for the study of endothelial cell function. Nonetheless, they may offer several practical advantages over primary cultures, including cellular homogeneity, extended life span, lack of requirement for serum and exogenous growth factors, and suitability for large-scale biochemical analyses and genetic manipulations. Specifically, the nontransformed SIE-F cells may have widespread utility in research on endothelial function in general, whereas owing to their ability to generate branching tubes in fibrin gels, SIE-S2 cells may provide a useful tool to investigate the factors that modulate the angiogenic process. Considering that the mouse is a frequently used experimental animal for studies on angiogenesis, arteriosclerosis, and thrombosis (Christofori and Hanahan, 1994; Carmeliet et al., 1998; Couffinhal et al., 1998), and that considerable differences may exist between endothelial cells of different species, the availability of clonal murine endothelial cell lines may facilitate the investigation of these and other pathophysiological processes.

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