The Moran Foundation
DEPARTMENT OF PATHOLOGY
BAYLOR COLLEGE OF MEDICINE
TEXAS MEDICAL CENTER
HOUSTON, TEXAS 77030

June 23, 1992

Gretchen J. Darlington, Ph.D.
Department of Pathology
Texas Children's Hospital
6621 Fannin
Houston, TX 77030

Dear Dr. Darlington:

Please update me on the status of your Moran Foundation project (3-90-0043) entitled "Sk-Hep - A Model for Angiogenesis".

Since approval and funding is generally for a one-year period, all projects approved in or prior to June 1991 should now be "complete", or nearly so.

I need a progress and/or final report regarding your project, including dates and times of any presentations, and information regarding any publications.

Please submit this to me within the next 30 days.

Sincerely yours,

Philip J. Migliore, M.D.
Research Director

PJM/ms

c: Dr. Michael Lieberman
Mr. John Moran

Texas Children's Hospital

BAYLOR COLLEGE OF MEDICINE

Department of Pathology

Located in the Texas Medical Center

6621 Fannin Street
MC 1-2261
Houston, Texas 77030-2399
713/770-2250
Fax: 713/770-1032

One Baylor Plaza
Texas Medical Center
Houston, Texas 77030-3498
713/798-4432

Milton J. Finegold, M.D.
Director of Laboratories
Gregory J. Buffone, Ph.D.
Associate Director of Laboratories
Dawna L. Armstrong, M.D.
Neuropathology
Gretchen J. Darlington, Ph.D.
Tissue Culture & Molecular Biology
Gail J. Demmler, M.D.
Director, Virology
Wm. Michael Dunne, Jr., Ph.D.
Director, Microbiology & Serology
Vicky Gresik, M.D.
Director, Hematology & Blood Bank
Edith P. Hawkins, M.D.
Renal & Immunopathology
Hal K. Hawkins, M.D., Ph.D.
Electron Microscopy
Debra L. Kearney, M.D.
Cardiovascular Pathology
Claire Langston, M.D.
Director, Anatomic Pathology
Ching-Nan Ou, Ph.D.
Director, Clinical Chemistry & Toxicology
Hannes Vogel, M.D.
Neuropathology

July 24, 1992

Philip J. Migliore, M.D.
Department of Pathology
Baylor College of Medicine

Dear Dr. Migliore,

A brief summary of the work carried out with the support of the Moran Foundation is attached. A manuscript describing the angiogenic cell line which we studied is also included.

The support of the Moran Foundation was instrumental in completing this work and Dr. Heffelfinger and I are grateful for the opportunity to pursue these studies.

With best regards,

Gretchen Darlington

Gretchen J. Darlington, Ph.D.

Summary of Studies on an Angiogenic Cell Line

Dr. Sue Heffelfinger, an Instructor in the Department of Pathology at Baylor, initiated studies on a human cell line which had been obtained by Dr. Darlington from a cell bank that described its origin as being from a hepatoma. Further characterization of the line demonstrated that it did not express hepatic gene products, but rather produced proteins characterisitic of endothelial cells. Because few established human endothelial cell lines were reported in the literature, Dr. Heffelfinger pursued the analysis of additional endothelial phenotypes of the cells *in vitro*.

Interestingly, she found that the cells formed capillary-like structures when they were provided specific substrata on which to grow. This cell line offers an opportunity to examine the genetic regulation of tubule formation. It may also be useful for the analysis of the second messenger signals that direct formation of capillary structures, and for an assessment of drugs that prevent or alter angiogenesis.

University of Cincinnati
Medical Center

Department of Pathology and
Laboratory Medicine

231 Bethesda Avenue
Cincinnati, Ohio 45267-0529
Phone (513) 558-4500
FAX (513) 558-2289

To: Gretchen Darlington

From: Sue Heffelfinger

Date: August 21, 1992

Re: Moran Foundation Grant

Final Report

Sufficient preliminary data on the interaction of SKHEP-1 clones with extracellular matrix proteins have been gathered to develop an R-29 grant proposal. The characterization of these clones is published in Heffelfinger SC, Hawkins HH, Barrish J, Taylor L, Darlington GJ. SKHEP-1: a human cell line of endothelial origin. *In Vitro. Cell. Devel. Biol.*, 28A:136-142, 1992, and Heffelfinger, SC, Barrish J, Hawkins H, Darlington G. SKHEP-1:a Model for Tubular Coalescence and Angiogenesis. *Lab Invest.* 1992, (in review). The presentation of these data was made at the 1991 Keystone Symposium, Heffelfinger SC, Darlington G.SKHEP-1: a model for angiogenesis. *J Cell Biochem* 15F(suppl): 250, 1991. Work on these clones is continuing in order to characterize the intracellular signaling mechanisms during vessel formation.

SH/ng

In Vitro Cell. Dev. Biol. 28A:136–142, February 1992

0883-8364/92 $01.50+0.00

SK HEP-1: A HUMAN CELL LINE OF ENDOTHELIAL ORIGIN

SUE C. HEFFELFINGER, HAL H. HAWKINS, JIM BARRISH, LINDA TAYLOR, AND GRETCHEN J. DARLINGTON

University of Cincinnati College of Medicine, Department of Pathology and Laboratory Medicine, 231 Bethesda Avenue, Cincinnati, Ohio 45267 and Baylor College of Medicine, Houston, Texas 77030

(Received 15 August 1991; accepted 10 October 1991)

SUMMARY

SK-HEP-1 is an immortal, human cell line derived from the ascitic fluid of a patient with adenocarcinoma of the liver. We have determined that these cells are of endothelial origin. Despite the location of the tumor from which SK HEP-1 was derived, the cell line does not have properties of hepatocytes. Northern blot analysis of total cellular RNA shows no messenger RNA for the hepatic-specific proteins albumin, alpha-fibrinogen, or gamma-fibrinogen. Endothelial characteristics are seen by transmission electron microscopy. These features include numerous pinocytotic vesicles, electron dense granules consistent with Weibel-Palade bodies, and abundant intermediate filaments, identified immunocytochemically as vimentin. Cultures grown on plastic dishes grow in bundles of polygonal to spindle-shaped cells. Proteins characteristic for endothelial cells are identified by immunocytochemistry. Addition of basement membrane material (Matrigel) or type I collagen to the cultures induces these cells to organize into a tubular network.

Key words: SK HEP-1; endothelium; Matrigel; tubule.

INTRODUCTION

SK HEP-1 is a permanent, human cell line derived from the ascitic fluid of a patient with a history of adenocarcinoma of the liver. This cell line was established in 1971 and has been reported to be of hepatocellular carcinoma origin. The original morphologic description was by Fogh and Trempe, 1975. Studies in nude mice (Fogh, 1977) and athymic rats and mice (Shouval, 1988) demonstrated the tumorigenicity of this cell line, as well as its sarcomatous pattern of growth. Turner and Turner, 1980, analyzed the secreted protein products of SK HEP-1 and showed that this cell line secreted alpha-1 antitrypsin and complement (C3), but no liver-specific protein products, such as albumin, alpha-fetoprotein, or haptoglobin. Libraries of monoclonal antibodies produced against this and other hepatocellular carcinoma cell lines, (Carlson, 1985; Shouval, 1985) have produced antibodies with reactivity for a subset of hepatocellular carcinoma cell lines, but no reactivity for adult or fetal liver. We have reexamined SK HEP-1 and determined that the cell of origin is endothelial.

MATERIALS AND METHODS

Cell culture. SK HEP-1 was kindly provided by the late Dr. Jorgen Fogh from the Sloan Kettering Institute of Cancer Research in 1975. Cells were grown in monolayer culture in M/M (three parts by volume Eagle's minimal essential media and one part Waymouth MAB 87/3) plus 8% bovine calf serum (Hyclone) and 2% donor horse serum (Hazleton). For induction of tubule formation, cells were plated on Matrigel (Collaborative Research) or type I collagen gels (Boehringer Mannheim). Human umbilical vein endothelial cells were the kind gift of Dr. C. Wayne Smith (Baylor College of Medicine). Clone SK refers to SK HEP-1 which has been in continuous culture in our laboratory and passaged weekly for several years.

Isolation of RNA. Cells were solubilized in guanidine HCl (Chirgwin et al., 1979) and RNA was isolated by the protocol of Glisin et al., 1974, or by utilizing the RNAZOL B method (CINNA/BIOTEX Laboratories).

Northern blot analysis. Total cellular RNA was electrophoresed essentially according to Maniatis et al., 1982. RNA was transferred onto Nytran for hybridization. Prehybridization of the filters was carried out for 4–18 hours at 42° C in 50% formamide, 5× SSC, 0.1% polyvinylpyrrolidone, 0.1% Ficoll, 0.1 mg/ml salmon sperm DNA, 20 m*M* sodium phosphate (pH 6.8), and 0.1% SDS. For hybridization the appropriate whole nick-translated plasmid or random-primed cDNA insert was added to this same mixture in addition to 10 mg/ml poly A, and 9.5 μg/ml poly C. Random primer labeling utilized an oligo labeling kit (Pharmacia). The filters were then washed in 2× SSC and 1% SDS twice at room temperature, 2× SSC and 1% SDS twice at 37° C, and 0.2× SSC and 1% SDS for 15 minutes at 65° C.

cDNA probes. We are grateful to the following investigators for the cDNA probes used in our studies. Human alpha-1 antitrypsin (Q631) was a gift from K. Matteson and A. Beaudet (Baylor college of Medicine, Houston, Texas; Matteson et al., 1982). The C3 probe was identified from an anonymous clone isolated from an acute phase liver cDNA library by 150 base pair sequence identity (DeBruijn et al., 1985). This cDNA (clone 15) was provided by S. Woo (Baylor College of Medicine, Houston, Texas). Alpha and gamma fibrinogen (p253) was a gift from S. Lord (University of North Carolina, Chapel Hill; Kant et al., 1983; Bolyard and Lord, 1988). The human albumin cDNA (F47) was obtained from R. Lawn (Genentech, Inc., South San Francisco, CA.; Lawn et al., 1981).

Immunohistochemistry. Cells were grown overnight at low density on multichambered slides (Lab-Tek), rinsed with phosphate buffered saline, fixed 10 minutes in 3% phosphate-buffered glutaraldehyde, followed by 0.1% Triton X permeabilization for 10 minutes. Prior to incubation with blocking serum, the cells were treated for 10 minutes in 0.3% H_2O_2 and 0.1% sodium azide. The following antibodies were purchased from Dakopatts: mouse monoclonal antihuman von Willebrand factor (clone F8/86), vimentin (clone V9), desmin (clone D33), HLA DR alpha (clone TAL.1B5), and epithelial membrane antigen (EMA, clone E29). Anti human von Willebrand factor was also purchased from AMAC (clone 4F9). Monoclonal antibodies to cytokeratins were from Hybritech (clone AE1:AE3) and anti-alpha smooth muscle actin (clone asm-1), from Boehringer Mannheim.

Three monoclonal antibodies to endothelial leukocyte adhesion molecule 1 (ELAM-1) were the kind gift of C. Wayne Smith (clone 2, 3, and 37). The human endothelial-specific monoclonal antibody, BMA 120, was the gift of A. G. Behringwerke, Marburg (Alles and Boslet, 1988; Totsch et al., 1990). The antibody reaction was visualized using the peroxidase mouse IgG, avidin-biotin kit from Vector Laboratories, Inc. Biotinylated *Ulex europaeus* agglutinin was purchased from Dakopatts. Peroxidase activity was developed using the chromogen 3-amino-9-ethyl carbazole or 3,3'-diaminobenzedine. Cells were counterstained using Meyer's hematoxylin or methyl green.

Karyotype. Cells were harvested by standard cytogenetic methods to obtain metaphase chromosomes. Slides were prepared by air-drying and chromosome banding was accomplished using a modified trypsin-Giemsa method (Seabright, 1971).

Histology. Cells cultured on Matrigel or type I collagen gels were removed from culture dishes with a spatula and embedded in 1% agar prior to routine histologic processing (37% formalin fixation and paraffin embedding).

Electron microscopy. Cell monolayers were fixed in situ in 3% phosphate-buffered glutaraldehyde for one hour, rinsed in buffer and post-fixed in 1% phosphate-buffered osmium tetroxide for one hour. The monolayers were dehydrated in the culture dish using ethanol, then separated from the dish using propylene oxide, washed in several changes of propylene oxide and embedded in Araldite 502 epoxy resin. Ultrathin sections were stained with lead citrate and uranium acetate and examined by electron microscopy.

Results

Hepatic-specific genes are not expressed by SK HEP-1. Hepatic-specific gene expression was examined by Northern blot analysis. Total cellular RNA was examined for the production of alpha and gamma fibrinogen, and albumin, which are produced only in the liver, and for alpha-1 antitrypsin and complement (C3), which are not hepatic-specific, but have been reported to be products of SK HEP-1 (Turner and Turner, 1980). Messages for albumin, alpha and gamma fibrinogen were not detectable (data not shown). Large amounts of complement (C3) message was seen (Figure 1 *a*). Alpha-1 antitrypsin message was found at a much lower level than the C3 message (Figure 1 *b*). Figure 1 *b* demonstrates that the amount of alpha-1 antitrypsin message is similar in SK HEP-1 and primary cultures of human umbilical vein endothelial cells (HEC), but much less than the mRNA level found in Hep 3B2, a human hepatoma cell line. Clone sk has very low levels of alpha-1 antitrypsin mRNA. These data are consistent with previously published protein data for this cell line (Turner and Turner, 1980). To confirm the presence of alpha-1 antitrypsin production, 24 hour SK HEP-1 conditioned media were analyzed by radioimmunoassay for alpha-1 antitrypsin (Kelly, 1989). Alpha-1 antitrypsin was secreted at a level of 4 $\times 10^{-5}$ ng/cell/24 hours.

SK HEP-1 is of human origin. The human origin of this cell line was confirmed by comparing a karyotype analysis of clone sk with previously published data (Turner and Turner, 1980). Twenty cells were analyzed. The modal chromosome number was 62 with a range of 56–64 chromosomes per cell (Figure 2). The karyotype showed several rearrangements and a notable absence of chromosome 13. These data are reasonably similar to the karyotype published by Turner and Turner for SK HEP-1.

SK HEP-1 clones show pleomorphic morphology. Multiple clones were grown in microtiter plates following dilution of recently thawed SK HEP-1 at Passages 6 and 12. All clones could be grown on untreated (non-protein coated) plastic. SK HEP-1 and isolated clones form a continuum in morphology on plastic dishes, from poorly adhesive, slowly dividing, highly irregular single cells to rapidly dividing, spindled cells which form a complete monolayer.

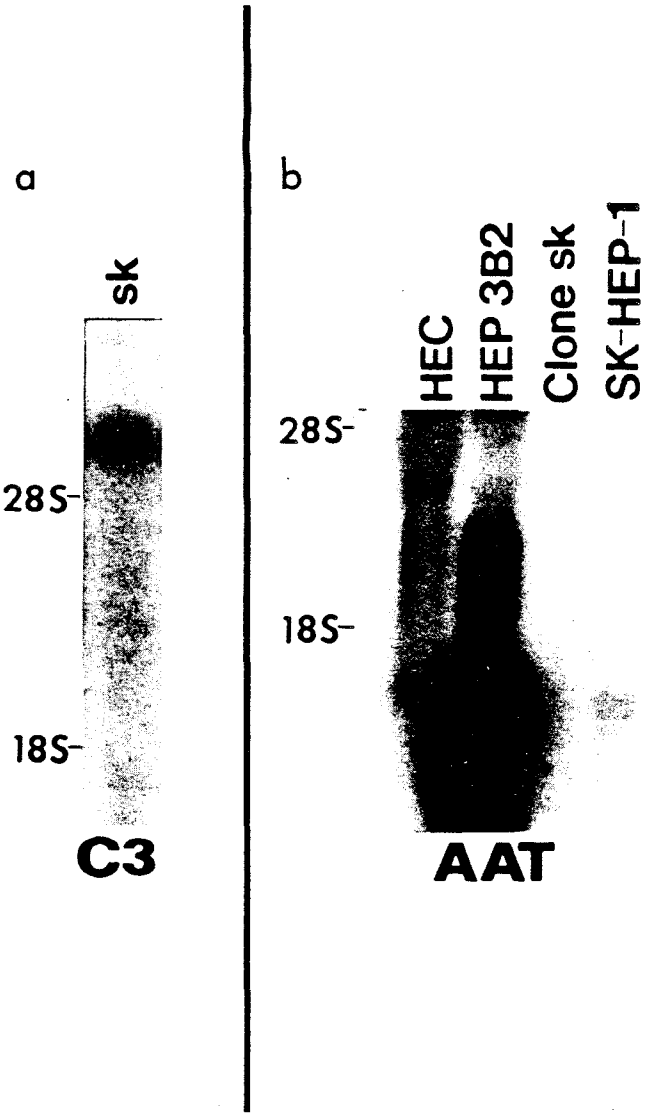

FIG. 1. Northern analysis of total cellular RNA. *a*) 20 μg RNA from clone sk hybridized to the cDNA for the human third component of complement. *b*) 20 μg of RNA per lane hybridized to the cDNA for alpha-1 antitrypsin. From *left* to *right*: human umbilical vein endothelial cells, Hep 3B2 (a human hepatoma cell line), clone sk, and SK HEP-1.

Many clones, which appear poorly adhesive, do not cover the entire flask, but grow off the surface on neighboring cells rather than grow to confluence as a monolayer. Indeed, some clones form large cell clusters on underlying flattened cells (Figure 3 *a*). Other clones form bundles of spindled cells which leave large areas of the flask uncovered. Over time in culture, small ingrowths of cells invade these spaces, eventually leading to a confluent flask (Figure 3 *b*). Individual cells of many clones have a distinct epithelioid morphology (Figure 3 *c*). Culture of cells at cloning densities showed that the cells were highly motile. This motility is demonstrated morphologically by the ruffled border of the leading edge (*arrow*, Figure 5 *a*) and the extended tail. Clones were isolated in microtiter plates because this high degree of motility prevented the development of independent colonies in flasks.

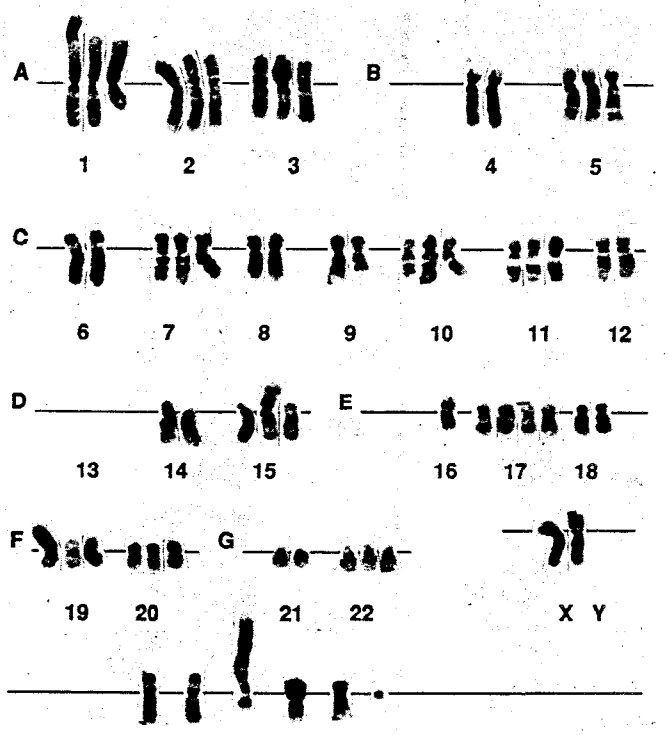

FIG. 2. Karyotype of clone sk. Marker chromosomes are placed in a row at the bottom.

SK HEP-1 has an endothelial ultrastructure. Ultrastructural study revealed that cells frequently came in contact near their ends and formed densities beneath the membranes of adjoining cells, consistent with tight junctions (Figure 4 *a*, *arrow*). The cytoplasm was simple, including elongated mitochondria, rough endoplasmic reticulum and Golgi apparatus. There were intermediate filaments throughout the cytoplasm (Figure 4 *b*). Numerous pinocytotic vesicles were clustered beneath the plasma membrane away from the flask surface (Figure 4 *a* and *c*). Figure 4 *c* also shows the presence of a coated pit. Occasionally, multiple small, electron-dense cylindrical bodies were present in the cytoplasm which were structurally consistent with Weibel-Palade bodies (Figure 4 *a*, higher magnification in Figure 4 *b*).

Immunohistochemistry confirms a mesenchymal origin. Table 1 shows a summary of immunohistochemical reactivity. Examination of SK HEP-1 with monoclonal antibodies to intermediate filaments vimentin, desmin, and the cytokeratins showed strong reaction to vimentin in all cells, no reaction to desmin, and a positive reaction to cytokeratins in a subpopulation of cells (data not shown). These data are consistent with a mesenchymal cell origin. To rule out a smooth muscle cell origin, SK HEP-1 was tested with anti-alpha actin. Reactivity with this antibody was slight to negative compared with cultured human fibroblast and arterial smooth muscle cell controls (data not shown). Two monoclonal antibody clones to the endothelial protein, von Willebrand factor, were reactive (clone 4F9, Figure 5 *a*). Three monoclonal antibody clones to endothelial-leukocyte adhesion molecule I were reactive in routinely cultured cells; reactivity to these antibodies was increased following four

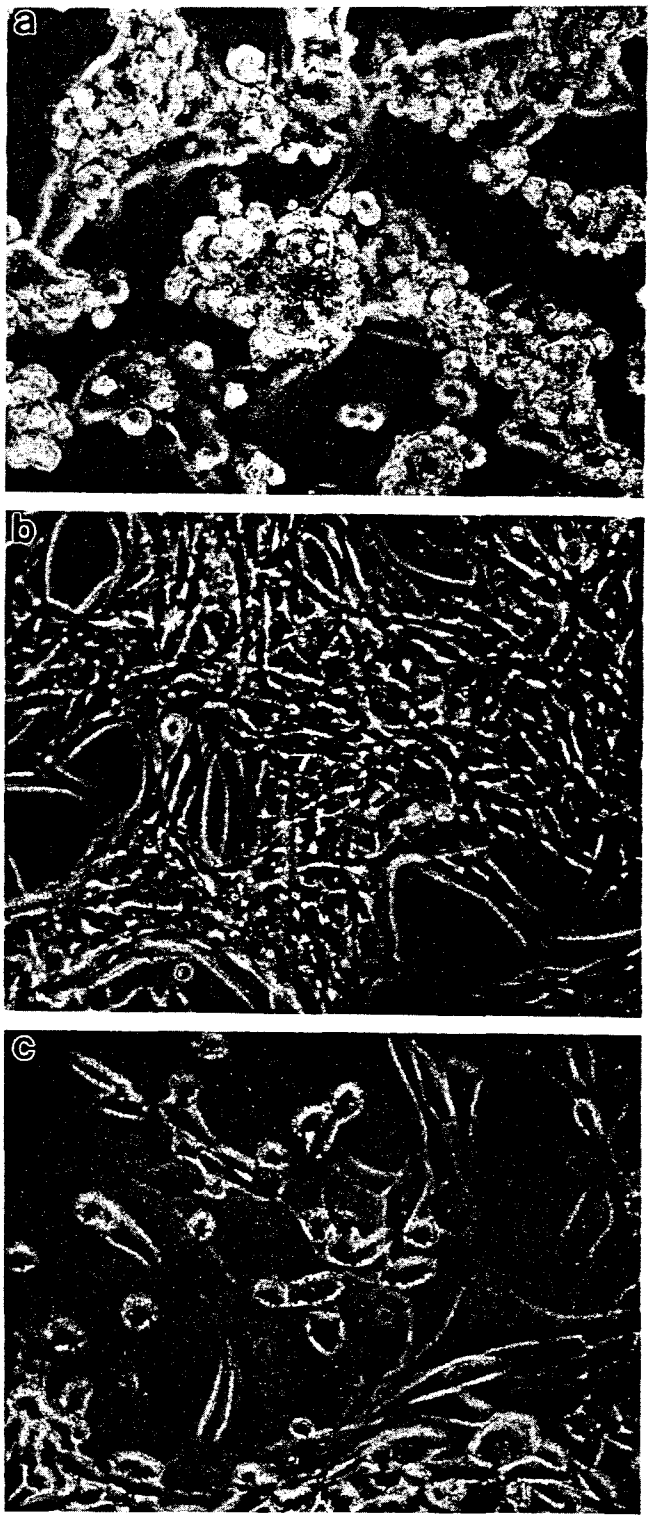

FIG. 3. Morphologic features of three clones derived from SK HEP-1 when cultured on uncoated plastic dishes. Under identical culture conditions, these clones have markedly different morphologies. The cells in *Panel a* adhere poorly to uncoated tissue culture dishes and tend to grow in aggregates on a few adherent cells. *Panel b* shows cells which grow in a layered crisscross pattern, similar to some smooth muscle cells. The clone shown in *Panel c* has an epithelioid morphology (×10, phase contrast).

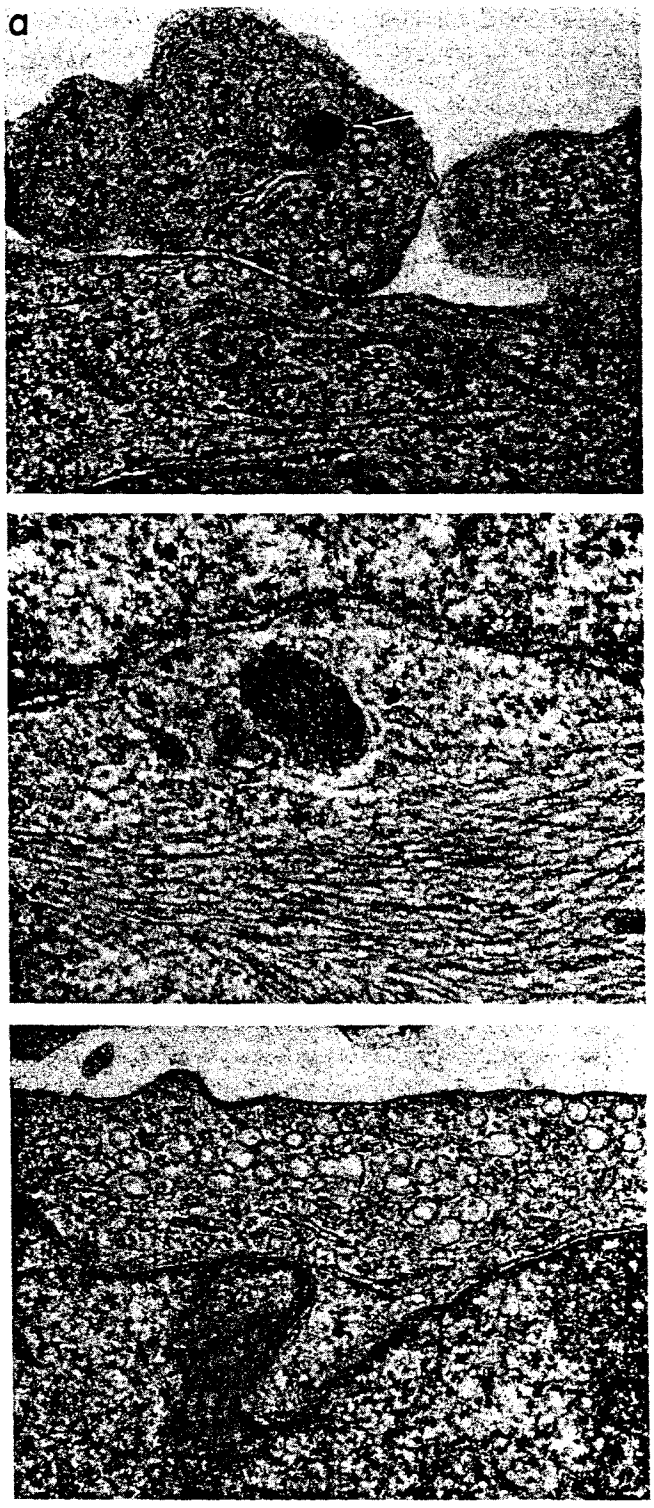

FIG. 4. Transmission electron microscopy of clone sk cultured on plastic *a*) Two cells are seen forming an intercellular junction, with electron dense material immediately beneath the plasma membrane. The upper cell contains pinocytotic vesicles and an electron dense organelle, *arrow* (×62 000). *b*) An increased magnification of the electron dense organelle, such as is seen in 4*a*. Numerous intermediate filaments are also seen (×72 000). *c*) Two cells which form an interdigitation. The upper cell has pinocytotic vesicles and a coated pit (×55 000).

TABLE 1

IMMUNOHISTOCHEMISTRY[a]

Pancytokeratin	Clonal
Desmin	−
Vimentin	+
Alpha actin	−
EMA[b]	−
von Willebrand factor	+
ELAM-1[c]	+
BMA 120	+
HLA DR alpha	−

[a] SK HEP-1 cells were cultured overnight on glass slides, rinsed with phosphate buffered saline, fixed, and permeabilized. Monoclonal antibody binding was demonstrated using an avidin-biotin complex-coupled horseradish peroxidase sandwich. A negative reaction with antibodies to HLA DR alpha was utilized as a control in all slide sets tested. The reaction with three monoclonal antibodies to ELAM-1 was positive without exogenous cytokine stimulation. However, other studies in our laboratory utilizing SK HEP-1 have demonstrated the production of Il-1 by these cells (unpublished data).

[b] Epithelial membrane antigen.

[c] Endothelial leukocyte adhesion molecule 1.

hours treatment with 10 ng/ml interleukin 1 β (Biogen, data not shown). In addition, these cells were reactive with the endothelial-specific antibody, BMA 120 (Figure 5 *b*). Negative controls included use of an IgG1 K isotype monoclonal antibody to HLA DR alpha (Figure 5 *c*) in each slide set and testing each primary antibody under identical conditions on the human hepatoma cell line, Hep 3B2. *Ulex europaeus* is a plant lectin commonly used as an endothelial marker in tissues. *Ulex europaeus* lectin was negative in SK HEP-1; we have not tested this lectin in cultured HEC's. The presence or absence of epithelial membrane antigen was examined to help rule out an epithelial origin. No reaction with antibodies to EMA was found. Hep 3B2 is positive for epithelial membrane antigen.

SK HEP-1 can undergo tubule formation. Primary cultures derived from micro or macro vascular endothelium can be induced to produce tubular structures under a variety of conditions (Maciag, 1982; Madri and Williams, 1983). Extracellular matrix proteins, particularly laminin, are important for this phenotype to be expressed (Kubota et al., 1988). To determine whether SK HEP-1 was capable of undergoing the morphologic change from monolayer to tubular structures, the cells from each clone were plated on a laminin-rich, basement membrane gel derived from the Engelbreth-Holm Swarm mouse tumor (Matrigel) or type I collagen gel. Within 48 hours of plating, the SK HEP-1 cells form a tubular network on the surface of Matrigel (Figure 6 *a*). Human umbilical vein endothelial cells form an identical tubular network on Matrigel (Figure 6 *b*). The time course of SK HEP-1 tubule formation was clone-dependent, being as short as 6 hours in one clone. Over time in culture, some tubules are produced under the surface of the gel. Cultures on type I collagen gels produce tubules with a less organized pattern (data not shown). Histologic sections show that these structures contain lumina, consistent with capillary formation. Figures 7 *a* and *b* show the histologic section of tubules grown within Matrigel and collagen gel, respectively. In both types of gels, the nucleus is flattened and the cytoplasm forms a tubular structure. Transmission electron microscopy of SK HEP-1 tubules within Matrigel also demonstrates a lumen (data not shown).

FIG. 5. Immunocytochemical analysis of SK HEP-1 utilizing antibodies against *a*) von Willebrand factor clone 4F9, *b*) BMA 120, and *c*) HLA DR alpha. (Meyer's hematoxylin counterstain, ×50). The *arrow* in *Panel a* points to a ruffled cell border, which is the leading edge of this actively motile cell.

DISCUSSION

SK HEP-1 is a permanent cell line derived from a patient with a history of adenocarcinoma of the liver. Consistent with previous studies, we find that this cell line produces no liver-specific proteins, such as albumin and fibrinogen, but does produce alpha-1 antitrypsin and C3. Alpha-1 antitrypsin is produced by many cell types including endothelial cells, as evidenced by the expression of alpha-1 antitrypsin mRNA in human umbilical vein endothelial cells. C3 also been shown to be synthesized by endothelial cells (Brooimans, 1990).

Since published histopathologic examination of SK HEP-1 in rodents and the in vitro growth pattern suggested a mesenchymal origin, we examined SK HEP-1 at the ultrastructural level. Most cells showed very poor differentiation. An abundance of intermediate filaments are identified immunocytochemically as vimentin. Many non-mesenchymal cells which are highly proliferative in vitro produced vimentin (Franke et al., 1979). However, the pattern of intermediate filament expression in SK HEP-1, i.e. the predominance of vimentin with rare cytokeratin reactivity, is most consistent with a mesenchymal origin. The presence of cytokeratin in a fraction of the cell population is also consistent with a wealth of literature on intermediate filament types in normal endothelium (Jahn,

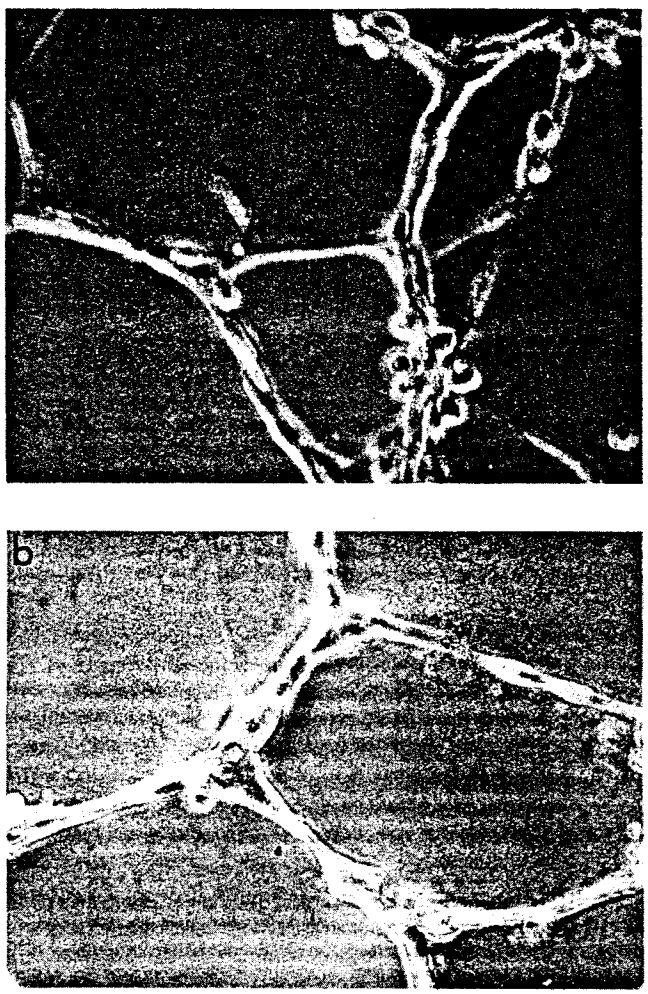

FIG. 6. Tubular structures produced by *a*) clone sk and *b*) human umbilical vein endothelial cells cultured for 48 hours on Matrigel (×10, phase contrast).

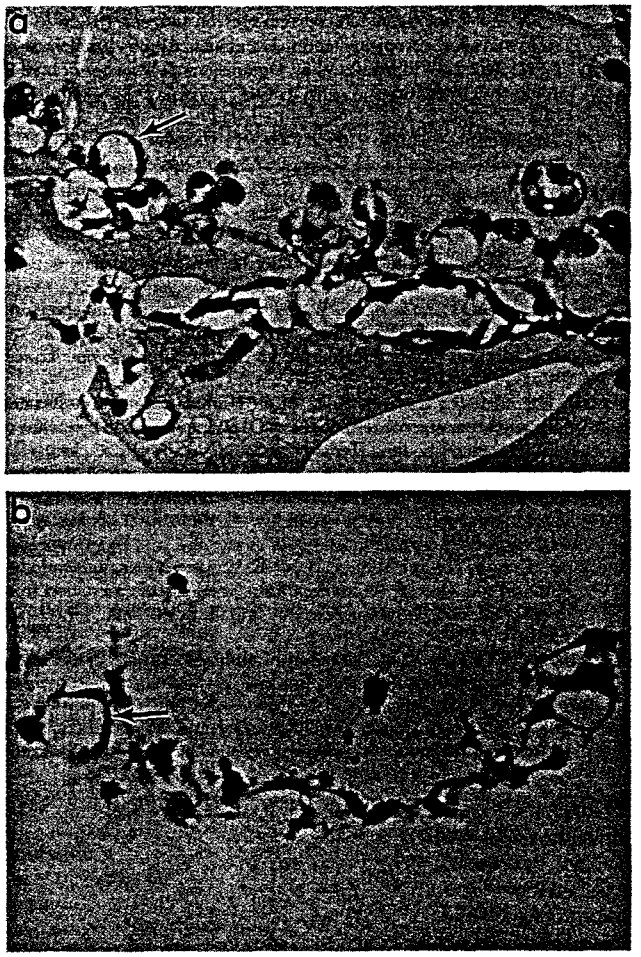

FIG. 7. Histologic examination of clone sk cultured on extracellular matrix gels. Clone sk was cultured for two weeks on *a*) Matrigel or *b*) type I collagen gel. The gel was embedded in 1% agar prior to fixation in formalin and paraffin embedding (hematoxylin and eosin; *a*, ×40; *b*, ×66). After a period of days, the cells, which are plated on the surface of the gel, divide and migrate into the gel as tubules. The arrows in each panel indicate tubular structures which clearly contain more than 1 nucleus. These nuclei are eccentric and flattened; the pale blue cytoplasm forms the perimeter of the tubule.

1987) and endothelial tumors (eg. Gray, 1990; Totsch, 1990). Negative stains for EMA and GFAP, both very sensitive markers for either epithelial or glial cells, also assured us that these cells were not from epithelial or glial tumors. The presence of pinocytotic vesicles at the surface of the cells, and organelles, structurally consistent with Weibel-Palade bodies, lead us to entertain an endothelial cell origin. The absence of binding to the *Ulex* lectin is not too surprising since *Ulex europaeus* lectin is also absent in some endothelial tumors (Wick, 1987; Alles, 1988). Finally, reaction with multiple monoclonal antibodies to von Willebrand factor, ELAM-1, and BMA 120 were used to demonstrate the endothelial nature of these cells.

Published studies of this cell line, as it produces tumors in animals (Shouval, 1988), show clearly that both spindled sarcomatous and whorled epithelioid patterns of growth can occur. We have confirmed the polyclonal nature of this cell line and shown that many clones do indeed appear epithelioid. We have found no correlation between morphology on plastic and staining for cytokeratins or von Willebrand factor. Published ultrastructural studies in these animal tumors show very poor differentiation. The biliary canaliculus reported in these studies, we believe to be cellular debris trapped between cells. Ultrastructural characteristics such as these, must be interpreted with caution.

The ability to produce tubular structures in vitro is a functional property of endothelial cells. Given an abundant and complex extracellular matrix substrate for growth, SK HEP-1 is able to produce capillary-like structures. The morphology of these cells in a monolayer and on Matrigel is very similar to the published morphology of primary human umbilical vein endothelial cell cultures (Grant et al., 1989) and our own umbilical vein endothelial cell cultures. Histologic and electron microscopic examination of these cells cultured on Matrigel confirms the presence of a lumen.

We have reexamined the morphologic features and protein products of the human cell line SK HEP-1 in order to determine the cell of origin. These types of studies are frequently difficult even when beginning with fresh tumor material. After nearly twenty years of manipulation in culture, many of the differentiated features can be lost. Early reports of this cell line indicated that there were no hepatic-specific protein products. These data are consistent with the experience in our laboratory, in which no hepatic features were found. Our recent data indicates that these cells retain some endothelial-specific properties, indicating a mesenchymal origin. Because some clones of this cell line are able to form tubules on extracellular matrix gels, we are continuing to study this cell line as a model for in vitro angiogenesis.

ACKNOWLEDGEMENTS

This work was supported in part by an American Liver Foundation fellowship (S. C. H.), a grant from the Moran Foundation #24-372250-142101, and NIH grant GM32111 (G. J. D.).

REFERENCES

Alles, J. U.; Bosslet, K. Immunocytochemistry of angiosarcomas: a study of 19 cases with special emphasis on the applicability of endothelial cell specific markers to routinely prepared tissues. Am. J. Clin. Pathol. 89:463–471; 1988.

Bolyard, M. G.; Lord, S. T. High-level expression of a functional human fibrinogen gamma chain in Escherichia coli. Gene 66:183–192; 1988.

Brooimans, R. A.; Van der Ark, A. A. J.; Buurman, W. A., et al. Differential regulation of complement factor H and C3 production in human umbilical vein endothelial cells by IFN-gamma IL-1. J. Immunol. 144:3835–3840; 1990.

Carlson, R. I.; Ben-Porath, E.; Shouval, D., et al. Antigenic characterization of human hepatocellular carcinoma; development of in vitro and in vivo immunoassays that use monoclonal antibodies. J. Clin. Invest. 76:40–51; 1985.

Chirgwin, J. M.; Przybyla, A. E.; MacDonald, R. J., et al. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294–5299; 1979.

DeBruijn, M. H. L.; Fey, G. H. Human complement component C3: cDNA coding sequence and derived primary structure. Proc. Natl. Acad. Sci. USA 82:708–712; 1985.

Fogh, J.; Fogh, J. M.; Orfeo, T. One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. Journal of the National Cancer Institute 59:221–226; 1977.