Virchows Arch (1995) 426:619–628
© Springer-Verlag 1995

ORIGINAL ARTICLE

Johanna Plendl · Fred Sinowatz · Robert Auerbach

A transformed murine myocardial vascular endothelial cell clone: characterization of cells in vitro and of tumours derived from the clone in situ

Received: 27 March 1995 / Accepted: 19 May 1995

Abstract In the course of maintaining a cloned murine myocardium-derived endothelial cell line (mouse heart endothelial cell clone 5; MHEC5) a spontaneously transformed variant has been identified (clone MHEC5-T). On injection into histocompatible mice, clone MHEC5-T uniformly generated epithelioid haemangioendotheliomas. Clone MHEC5-T underwent significant additional alterations in addition to the acquisition of tumour-forming potential in vivo along with the diagnostic correlate of loss of cellular contact inhibition in vitro. Whereas the transformed cells maintained lectin-binding properties characteristic of endothelial cells, they lost the cell surface receptor(s) for acetylated low density lipoprotein and no longer bound antibodies to either angiotensin converting enzyme or von Willebrand factor-associated antigen. Vascular cell adhesion molecule-1 (VCAM-1), expressed constitutively on the parent clone, was down-regulated in the transformed cell line. The transformed cells acquired immunoreactivity to antibodies directed against cytokeratin, and they showed a markedly increased response to migration-inducing factors in vitro. The cell line described in this report demonstrates that the in vitro transformation of myocardium-derived endothelial cells can lead through transitional stages of differentiation to a new stable phenotype characterized by endothelial – to – epithelioid transition. The study of MHEC5-T cells, in addition to providing insight into the biology of cardiac neoplasms, may help to elucidate regulatory mechanisms involved in endothelial cell activation, transition and transformation.

Key words Epithelioid haemangioendothelioma · Heart neoplasms · Cell line · Flow cytometry · Mouse

J. Plendl (✉) · F. Sinowatz
Institute for Veterinary Anatomy, University of Munich, Veterinärstrasse 13, D-80539 München, Germany

R. Auerbach
Center of Developmental Biology, University of Wisconsin, W. Johnson Street Madison, Wisconsin 53706, USA

Introduction

The incidence of primary heart tumours is low when compared with those in other organ sites such as the lung, colon, bladder or breast. However, when these tumours occur they are most frequently of vascular origin [23, 25, 28, 49]. Immunohistochemical and ultrastructural evidence indicates that the differentiation of a cardiac vascular tumour may progress along three cell differentiation pathways: smooth muscle, fibroblastic and endothelial [15, 50].

Although epithelioid haemangioendothelioma is a rare vascular tumour [12, 30] it may prove particularly useful in tracing the developmental changes leading from normal endothelial cells to neoplastic ones. These tumours mimic a number of soft tissue tumours of diverse non-endothelial phenotypes, and thus indicate that endothelial cells have the potential to differentiate along several developmental pathways. For example, these tumours contain an abundance of cytokeratin and vimentin, in addition to manifesting a more stellate morphology than typical haemangioendotheliomas [22].

A major factor in the dearth of information concerning the nature of the endothelial component of cardiac tumours can be ascribed to a lack of suitable cell culture systems as well as the absence of a useful animal model which lends itself to experimental analysis. The myocardium-derived epithelioid haemangioendothelioma cell line which we describe may help initiate more extensive studies into the origin and pattern of differentiation of heart neoplasms.

Materials and methods

NMRI mice, a Swiss-albino-derived strain, were obtained from the GSF-Forschungszentrum für Umwelt und Gesundheit, München-Neuherberg, Germany. The NMRI/GSF mice have their origin in the Swiss mice of the Naval Medical Research Institute (Bethesda, Md., USA), and were obtained by the GSF from Ivanov, Isny, in 1966. Although these animals have been maintained as an outbred colony since that time and thus are nominally not inbred, skin and tumour transplantation analysis as well as mixed leucocyte reac-

tion tests have shown them to be a line of histocompatible mice (unpublished observations). Their major histocompatibility complex haplotype has not, however, been determined.

Cells were isolated as described elsewhere [41]. In short, hearts of 2-week-old animals were removed aseptically. The myocardium was separated from pericardium, epicardium and endocardium. Myocardium was minced with scissors and washed in 0.01 M phosphate buffered saline (PBS), pH 7.2. The pellet was incubated at 37° C in 0.5% collagenase (Sigma, St. Louis, USA). After 30 min the suspension was washed in PBS, centrifuged and the pellet was resuspended in primary medium, which consisted of Dulbecco's modification of Eagle's minimum essential medium (DMEM; Sigma) with 20% fetal bovine serum (FBS; Sigma), 100 U/ml penicillin, 100 µg/ml streptomycin, 20% S180-tumour-conditioned medium, 5 U/ml heparin (Sigma), 50 µg/ml endothelial cell growth supplement (ECGS; Collaborative Biomedical Products, Bedford, Mass., USA) and 1% basal medium Eagle vitamin solution (Sigma). The cell suspension was filtered through a series of Nitex filters with pores from 200 µm to 15 µm. Subsequently cells were seeded into six-well culture dishes precoated with 1.5% gelatin (Sigma). After 15 min all non-adherent cells were removed by gently washing the plates with medium and adherent cells were fed primary medium to selectively support growth of endothelial cells. After expanding, cells were cultured in 100 mm culture dishes and passaged after reaching confluency. After passage 5, the concentration of ECGS was reduced to 5 µg/ml, the amount of FBS was lowered to 10% FBS, and the addition of S180-tumour conditioned medium was discontinued.

The mouse heart endothelial cell clone 5 (MHEC5) was one of several clones derived from the parent cell line at passage 25 by limiting dilution. Inasmuch as cells exhibited a cloning efficiency of 10%–25%, cells seeded at a density of one cell per well in 96-well-plates were used to achieve single cell clones.

Endothelial cells were identified on the basis of morphology, binding affinity for the monoclonal antibody to angiotensin converting enzyme (α-ACE 3.1.1), production of functionally active ACE by tripeptide cleavage assay (Vector Laboratories, Burlingame, Calif., USA), labelling with rabbit anti-human von Willebrand factor, vWF (Dako, Hamburg, Germany) and uptake of 1,1'dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanine perchlorate acetylated density lipoprotein (acLDL), dilacLDL (prepared by Dr. Daniel G. Malone, University of Wisconsin, Madison, USA). Standard protocols for these tests have been described in detail in our previous publications [21, 37, 41].

Karyotyping was performed according to standard methods. Briefly, cells were incubated overnight in medium containing 0.2 µg/ml Colcemid (Gibco, Grand Islands, N.Y., USA), trypsinized, washed and suspended in 0.075 M potassium chloride. After fixation in methanol/acetic acid (1:2), cell suspensions were dropped onto a slide, stained with Giemsa and examined under oil immersion.

For electron microscopy, cells grown on coverslips were washed thoroughly with PBS and fixed overnight by immersion with Karnovsky's fixative at 4° C. After three washes, postfixation was performed with 2% osmium tetroxide at 4° C. The samples were dehydrated in an ethanol series and embedded in vinyl cyclohexene dioxide, ERL 4206 (Agar Scientific, UK). Polymerization was done at 70° C for 24 h. Ultrathin sections (50–60 nm) were cut on a LKB ultramicrotome with a diamond knife, mounted on copper grids and contrasted with uranylacetate (10 min) and lead citrate (5 min). Sections were examined with a Zeiss 902 electron microscope.

To undertake lectin staining cells were removed from culture by brief ethylenediaminetetracetic acid (EDTA) treatment. Lectins (*Dolichos biflorus* agglutinin, DBA; *Bandeiraea simplicifolia* lectin I, BSL I; Concanavalin A, Con A; *Ulex europaeus* agglutinin I, UEA I; all labelled with fluorescein isothiocyanate, FITC) were obtained from Vector and used at a final concentration of 2.5 µg/ml in PBS/azide (0.1%).

Immunocytochemistry was performed on cells detached from culture dishes by brief EDTA treatment. They were rinsed with PBS and α-VCAM, a rat monoclonal antibody [33] (obtained from Dr. Paul Kincade, University of Oklahoma, USA) was used at a concentration of 1:1,000 in PBS to label live cells. A goat anti-rat IgG-FITC (Sigma; 1:400) was used as a second-step reagent.

For intracellular labelling, cells were fixed with methanol-acetone (1:1) for 5 min at 4° C. Cells were incubated with α-cytokeratin (1:50 in PBS) or α-vimentin (1:200 in PBS; both from Sigma), for 45 min at 4° C. FITC-labelled goat-anti mouse IgG (1:100 in PBS), was used as second-step reagent.

Flow cytometric analysis was carried out using a Becton-Dickinson (Mountain View, Calif., USA) FACScan instrument (15 mW argon ion laser, 488 nm, 200–220 µm nozzle aperture, 400–1000 cells/s flow rate). Data were analysed using the LYSISII software programme provided by Becton-Dickinson. Live gating to exclude dead cells was used on the basis of their uptake of propidium iodide.

Analysis of cell movement (chemokinesis) was carried out in microtiter plates, using an image analysis programme to obtain quantitation of migration tracks made by displacement of indicator microspheres (0.93 microspheres; Polysciences, Warrington, Pa., USA) as described previously [36]. In brief, microbeads were suspended in DMEM, sonicated to break aggregates, then added to 96-well-plates preincubated with 500 µg/ml ovalbumin. Plates were centrifuged for 25 min at 500×g. Culture medium containing different concentrations of FBS (0.5%, 1%, 2.5%, 5%, 10%; Sigma) was added. Cells were adjusted to a concentration of 10^4 cells/ml and 500 cells were added to each well. Cultures were examined at intervals from 3 h to 48 h.

Cells of clone MHEC5-T as well as of the untransformed clone MHEC5 were injected subcutaneously (10^6 cells in 0.1 ml PBS/animal) or intravenously (10^5 cells in 0.02 ml PBS/animal) into 1-day-old (n=10) and 5-week-old (n=10) mice.

For light microscopy, tumours were fixed in 4% formalin or in methanol-acetic acid (1:2) and embedded in paraffin. Sections 6 µm thick were stained with haematoxylin and eosin. For electron microscopy, tumours were fixed in Karnovsky's fixative and subsequently treated as described for cell cultures (above).

Sections of tumours were labelled with the following antibodies: α-vWF (rabbit anti-human; 1:100 in PBS, after trypsinization for 20 min at 37° C; Dako) or α-vimentin (monoclonal anti-mouse, 1:40 in PBS; Sigma) or α-cytokeratin (monoclonal anti-mouse, 1:400 in PBS, after protease-incubation; Sigma). Paraffin sections were deparaffinized, incubated with primary antibody overnight at 4° C, washed in PBS and incubated with a horseradish peroxidase (HRP) conjugate of second-step antibody at room temperature for 2 h.

Lectin labelling of sections was achieved using HRP conjugates of lectins (Sigma) at a concentration of 25 µg/ml. Deparaffinized sections were treated with 0.2% hydrogen peroxide to destroy endogenous peroxidase activity, washed in PBS, incubated with lectin for 45 min at room temperature in a humidified chamber, washed, and incubated in 3,3'-diaminobenzidine. Subsequently slides were washed, dehydrated and mounted. A selection of slides was counterstained with cresyl violet. Technical details have been published previously [39, 40].

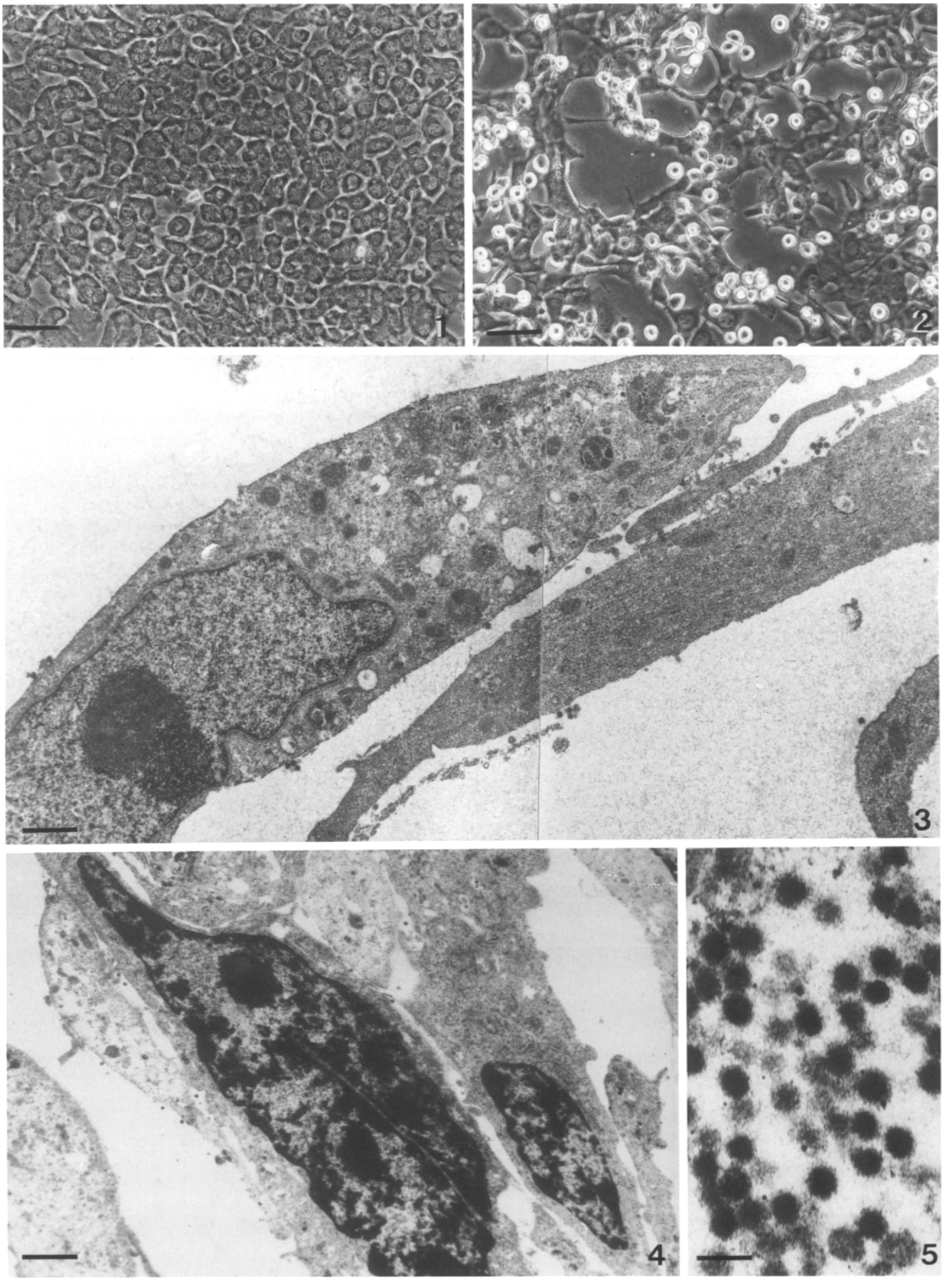
Fig. 1 Mouse heart endothelial cell clone 5 (MHEC5) cells in vitro, phase contrast. Confluent monolayer with typical cobblestone appearance. ×480, *Bar*=38 µm

Fig. 2 Transformed (MHEC5-T) cells in vitro, phase contrast. After transformation, cells grow in reticular structure, round up and pile up. ×480, *Bar*=38 µm

Fig. 3 MHEC5 cells in vitro, electron microscopy. Cells show regular contours of nuclei. No Weibel-Palade bodies can be found. ×8,000, *Bar*=1 µm

Fig. 4 MHEC5-T cells in vitro, electron microscopy. Cells show irregular contours of nuclei, large number of elongated mitochondria and dilated endoplasmatic reticulum. Weibel Palade bodies are absent. ×8,000, *Bar*=1 µm

Fig. 5 MHEC5-T cells in vitro, electron microscopy. Groups of viruses characterized by dense cores. Their appearance is similar to those seen in MHEC5 cells. ×45,000, *Bar*=0.1 µm


1
2
3
4
5

Results

During the first 41 passages the cells grew steadily but slowly, exhibiting typical cobblestone morphology on confluence (Fig. 1). They were split in a 1:5 ratio every 2 weeks, suggesting an average doubling time of 72 h. At passage 42 the proliferation and growth pattern changed dramatically. The splitting rate had to be changed to one to two weekly passages at a ratio of 1:20 (average doubling time <20 h). The cells grew in a reticular structure and lost contact inhibition as shown by rounding up and piling up of cells (Fig. 2). About one-third of the round cells detached from the culture dish and grew in suspension. However, these cells retained reproductive potential, giving rise to both adherent and subsequent non-adherent cells on transfer. The transformed cells were not dependent on the addition of ECGS to the culture medium.

Electron microscopy showed that transformed cells were pleomorphic, some elongated, others in plump shapes in contrast to the round or oval shape of the non-transformed cells. Irregular contours of nuclei with clefts and invaginations were apparent and mitotic figures were frequent. Compared with the non-transformed MHEC5 clone, the MHEC5-T cells harboured a large number of elongated mitochondria and dilated endoplasmatic reticulum. Extensive search failed to reveal Weibel-Palade bodies in any of the cells (clone MHEC5 or clone MHEC5-T). No microtubules, no microfilaments and no intermediate filaments were observed (Figs. 3, 4). The cell membrane revealed a large number of microvilli-like structures. Intercellular contacts between the transformed cells were unusual: desmosomal-like junctional complexes extending over a long distance of the cell membrane were noticed, suggesting functional relevance. Interdigitations between cells were frequent. No such specific membrane structures could be seen in the non-transformed MHEC5 cell line.

Ultrastructural evidence for viral replication was detected in both transformed and non-transformed cells (Fig. 5). Production of viral bodies could be traced back to passage 4 (the earliest passage for which cells were still available) of the non-cloned cell line from which MHEC5 was derived. Viruses were characterized by a round, dense core. Groups of viral bodies accumulated in vacuole-like structures. Budding of viruses and viruses in intercellular areas could be observed.

Endothelial cells became aneuploid as early as passage 4 (before the cells were cloned) and 38 passages before transformation occurred. Most cells contained a near-tetraploid number of chromosomes. Such karyotypic changes are typically observed in established murine microvascular and macrovascular endothelial cell cultures (unpublished observations).

Whereas the non-transformed MHEC5 cells bound the monoclonal antibody to ACE, the transformed cell line did not label with this antibody (Figs. 6a, b, c; 7a, b, c). Similar results were obtained with the tripeptide cleavage assay for functional enzyme activity: the transformed cells were unable to cleave hippuric acid-glycine-glycine, in contrast to the parental MHEC5 cells. Similarly, cloned MHEC5-T cells, in contrast to the parental non-transformed cell line, failed to stain with antibodies to vWF.

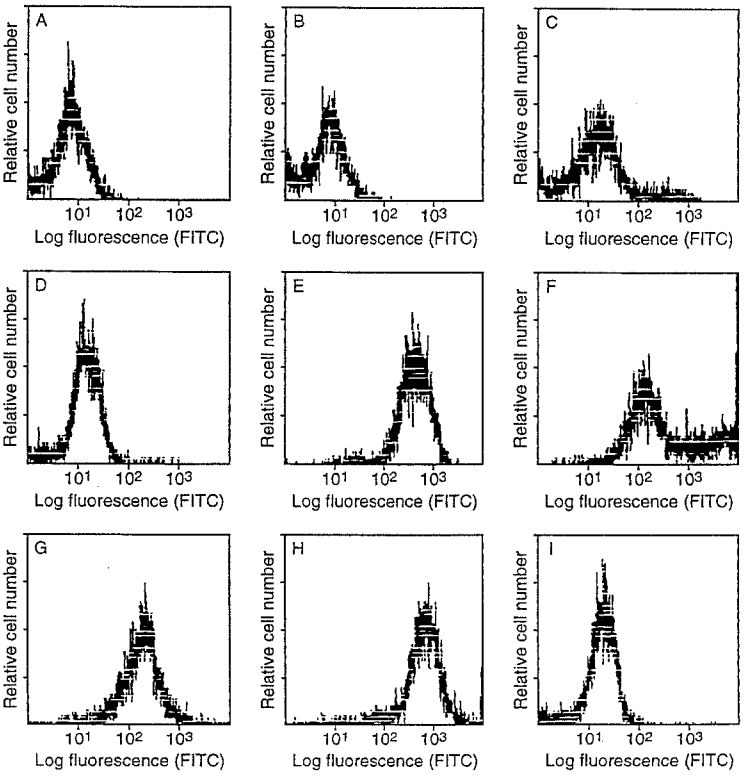


Fig. 6A–I Flow cytometric analysis of MHEC5. Not all data were obtained in the same experiment, but all settings were standardized and the instrument calibrated weekly. **A** Unlabelled cells, passage 45. **B** Second-step anti-mouse-IgM-fluorescein isothiocyanate (FITC) only. **C** Anti-angiotensin converting enzyme (ACE)+anti-mouse-IgM-FITC. **D** Second-step anti-rat IgG-FITC only. **E** Anti-vascular cell adhesion molecule (VCAM)+anti-rat IgG-FITC. **F** Lectin *Dolichos biflorus* agglutinin (DBA)- FITC. **G** *Bandeiraea simplicifolia* lectin 1 (BSL I)-FITC. **H** Lectin Concanavalin A (Con A)-FITC. **I** Lectin Ulex europaeus agglutinin I (UEA I)-FITC

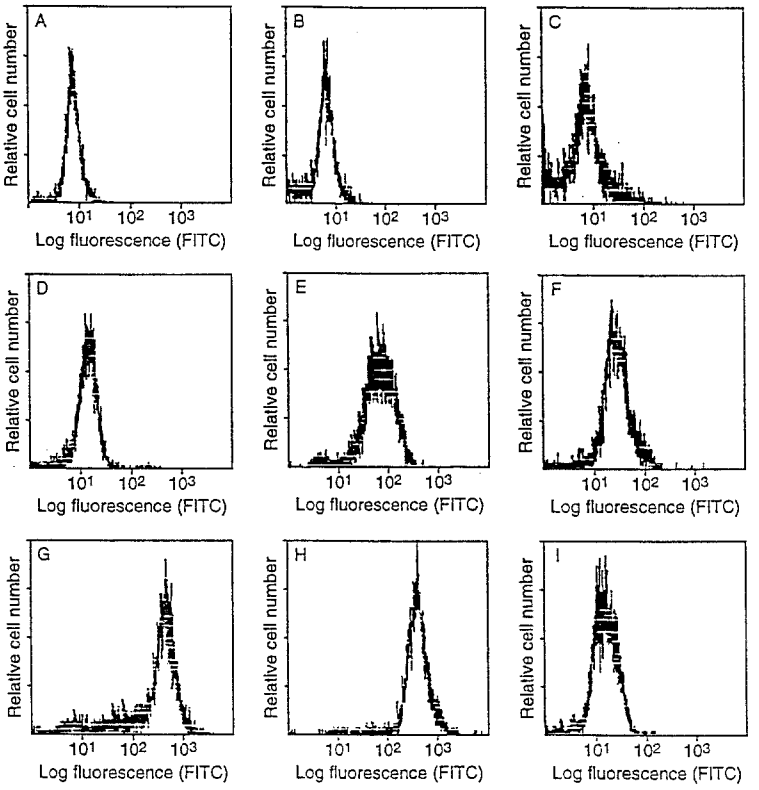


Fig. 7A–I Flow cytometric analysis of transformed cells, MHEC5-T. **A** Unlabelled cells, passage 45. **B** Second-step anti-mouse-IgM-FITC only. **C** Anti-ACE+anti-mouse-IgM-FITC. **D** Second-step anti-rat IgG-FITC only. **E** Anti-VCAM+anti-rat IgG-FITC. **F** Lectin DBA-FITC. **G** Lectin BSL I-FITC. **H** Lectin Con A-FITC. **I** Lectin UEA-I-FITC

Cloned MHEC5-T cells showed no binding or internalization of dilacLDL even after incubation for 18 h. This contrasts with the behaviour of the non-transformed cell line, which showed significant uptake of this reagent.

When labelled with antibodies against vascular cell adhesion molecule-1 (VCAM-1), both the transformed and parental cells were able to bind this antibody. However, the intensity of staining was reduced in MHEC5-T (Fig. 7d, e) cells when compared with the original untransformed cells (Fig. 6d, e).

There was a marked change in the affinity shown by the transformed versus non-transformed cells for antibodies to cytokeratin and vimentin. Whereas the parental MHEC5 cells labelled only with α-vimentin antibodies, the transformed cell line expressed both vimentin and cytokeratin. A small population of the transformed cells failed to bind either of the antibodies.

Concerning lectin binding only marginal differences were observed between the two cell lines. MHEC5-T clearly expressed the endothelium-specific DBA binding of the NMRI strain of mice, but staining intensity was reduced (Figs. 6f, 7f). Differences in extent of binding between the clones was also apparent for BSL I, with the transformed cells showing a more intense labelling than the parental MHEC5 cells (Figs. 6g, 7g). No differences could be found for Con A (positive for both cells lines; Figs. 6h, 7h) or UEA I (low binding affinity; Figs. 6i, 7i).

A distinct difference in the migration potential of MHEC5-T versus MHEC5 was observed. The time of onset of migration was much more rapid in the transformed cell line compared to the non-transformed one. By as little as 4 h marked migration tracks of MHEC5-T could be observed, in contrast to MHEC5 cells which even after 24 h showed only modest movement. Differences between the cell lines were seen at all serum concentrations and persisted over a 48 h period (Fig. 8a, b).

In vivo all animals injected at either 1 day (n=10) or 5 weeks (n=10) with MHEC5-T cells developed tumours, whereas no animal injected with the untransformed MHEC5 cells developed such tumours after more than 3 months. Animals injected subcutaneously developed palpable tumours at the injection site within 1 week. Intravenously injected animals developed multiple lung tumours. One of the ten animals injected intravenously developed a subcutaneous tumour in the lateral thoracic region in addition to the lung tumours.

Subcutaneous tumours grew as solid nodules of white-yellowish colour interspersed with dark, haemorrhagic areas. Lung tumours grew as nodules of varying diameter up to about 2 mm. Tumour growth was invasive: subcutaneous tumours invaded skeletal muscle; lung tumours invaded the thymus (Fig. 9). Whether the animal with both a subcutaneous and lung tumour should be considered to have multiple primary tumours or metastatic spread is not clear.

Histologic examination showed that small parts of the tumours were composed of cyst-like, blood-filled spaces lined by flat endothelial cells (Fig. 10). The largest part, however, was not as clearly vasoformative, but revealed proliferation of large polygonal cells with abundant cytoplasm, pleomorphic nuclei with vacuoles, distinct nucle-

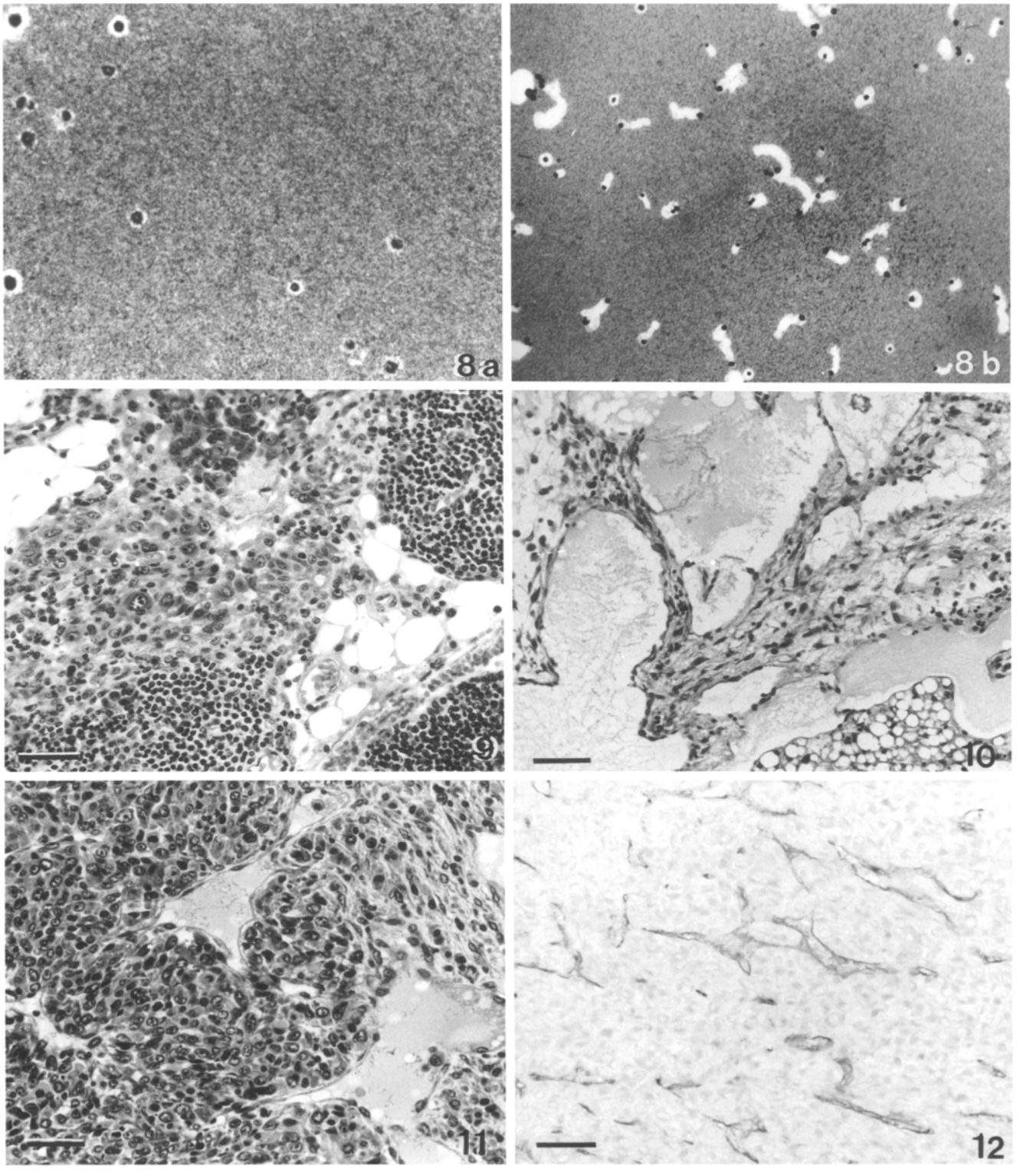


Fig. 8a, b Cell migration assay. Chemokinetic tracks 4 h after cell seeding. **a** Non-transformed cells, MHEC5. **b** Transformed cells, MHEC5-T

Fig. 9 Tumour in situ, generated by injection of MHEC5-T cells. Tumour cells have invaded the thymus, presumably from a large primary that had developed in the lung. Haematoxylin and eosin stain, ×400, *bar*=50 μm

Fig. 10 Angioformative region of tumours shows cyst-like blood filled spaces lined by endothelial cells. Haematoxylin and eosin stain, ×400, *bar*=50 μm

Fig. 11 Epithelioid region of tumours shows cells arranged in cords and nests surrounding narrow, blood-filled spaces. Capillaries are lined by plump, endothelial cells. Haematoxylin and eosin stain, ×400, *bar*=50 μm

Fig. 12 Section of tumour stained with peroxidase-coupled DBA lectin. The endothelium of vessels within the tumour labels strongly. Tumour cells in epithelioid areas show moderate staining, ×400, *bar*=50 μm

oli and frequent mitotic figures (around 15 in ten high power fields). Cells were arranged in cords, clusters or nests, surrounded by narrow blood-filled spaces, suggesting vasoformative properties (Fig. 11). Capillary-like vessels were lined by plump epithelioid endothelium (Fig. 11). Cells resembled malignant epithelial cells morphologically and were thus diagnosed as epithelioid haemangioendotheliomas.

Electron microscopy revealed large, irregularly-indented nuclei containing more than one nucleolus. Intercellular junctions were desmosome-like, similar to the in vitro situation. No intermediate filaments and no Weibel-Palade bodies were seen. Viruses were found within the cytoplasm as well as in the intercellular areas.

Endothelium of normal blood vessels in the lung labelled strongly with α-vWF (anti-factor VIII-related antigen). Endothelium of vessels in other organs examined was labelled discontinuously (skin) or was negative (heart, thymus) when stained with α-vWF. No blood vessels within the tumours, nor tumour cells, were labelled and only a few blood capillaries in the tumour periphery showed a reaction.

Cells of the epithelioid parts of the tumours were labelled homogeneously with α-vimentin, but labelling was very weak. Labelling with α-cytokeratin was seen in a few epithelioid cells only and was also weak.

Endothelial cells of normal blood vessels were labelled with the lectin DBA. Endothelium of all capillaries within the tumours was labelled strongly and tumour cells weakly. The endothelium of the large, cyst-like, blood-filled spaces was also labelled (Fig. 12). Normal vascular endothelium bound BSL I, but the endothelium of intratumourous vessels was negative. The cytoplasm of the majority of the tumour cells was clearly BSL I-positive, whereas nuclei were lectin-negative. Tumour cells found invading neighbouring tissue displayed a most intense affinity for DBA and BSL I. No labelling was seen with either Con A or UEA I.

Discussion

We have examined a transformed murine myocardium-derived vascular endothelial cell clone (MHEC5-T) showing a transformed cell phenotype in vitro and neoplastic growth in vivo. Tumours produced by the transformed cells were classified as epithelioid haemangioendotheliomas. To the best of our knowledge, MHEC5-T is the first established tumorigenic endothelial cell line of myocardial origin and the first epithelioid haemangioendothelioma which has been propagated and analysed.

Ultrastructural evidence for viral replication was found in both non-transformed and transformed cells, and could be traced back at least to passage 4 (more than 100 cell doublings before the appearance of the transformed phenotype). Viral infection of endothelium can lead to a wide range of effects [1]. For example, vascular neoplasms such as haemangiomas and haemangiosarcomas can be induced by different murine sarcoma, polyoma and papova viruses [9, 14, 17, 38, 58]. It has been proposed that Kaposi's sarcoma, believed to be of vascular endothelial origin, is induced by an AIDS-associated virus [45]. To date a viral aetiology for epithelioid haemangioendothelioma has not been established. Although we cannot exclude the possibility that the viruses detected in MHEC5-T played a role in transformation, the similarity between the morphology of viruses of the early myocardial endothelial cell cultures of MHEC5-T suggests that they were not of primary importance.

Although cobblestone morphology has long been considered a hallmark of endothelial cell cultures, it should be recognized that the cobblestone pattern is not characteristic for all endothelial cells and that there is considerable heterogeneity in the appearance of endothelial cells in vitro [4, 5, 6, 59]. Moreover, it is only at reaching confluence that this pattern becomes established. Thus cells that are not contact-inhibited or sprouting endothelial cells such as those involved in new vessel formation cannot be expected to manifest cobblestone-type organization.

It is now generally recognized that there is no single property that identifies an isolated cell as being an endothelial cell. For example, not all endothelial cells produce ACE or receptors for acLDL and there is variability of expression of vWF/factor VIII-related antigen expression even among endothelial cells of a single vessel. In addition, there is considerable variability in the expression of such cell surface markers as CD31, CD34 and receptors for growth factors such as basic fibroblast growth factor or vascular endothelial growth factor [2, 4]. Similarly, there is much variability in the array of cell surface-associated adhesion-mediating molecules. Some endothelial cell lines show a constitutive presence of VCAM, whereas others develop cell surface VCAM expression only following activation by cytokines or not at all [33], and there are major differences in glycoconjugate expression as evidenced by lectin binding properties and cell-cell interactions between endothelial cells and specific tumour cell types [2, 3, 11, 34, 35]. Seen in this context, it is not surprising that transformation was accompanied by changes in serologically detectable vWF/factor VIII-associated antigen or receptors for ac-LDL or in the production of ACE.

The endothelial nature of MHEC5-T is shown in vitro as well as in situ by labelling with two different endothelium-specific lectins: DBA and BSL 1. Vascular endothelium including the heart endothelium of certain mouse strains like the NMRI mouse exhibits a specific affinity for the lectin DBA [39, 40, 42, 43]. When compared to the non-neoplastic counterpart DBA binding is downregulated in MHEC5-T cells, but these cells still show specific binding of this lectin, as do the tumours derived from injection of the cells into mice. Strikingly, cells not included in the epithelioid part of the tumour but exhibiting an invading and migrating (angiogenic?) phenotype, labelled intensely with the DBA lectin as well as with BSL I. This finding is consistent with studies of Williams et al. [57] who described the angiogenic phenotype

of endothelial cells to be DBA-positive. BSL 1 has been demonstrated to label murine endothelial cells specifically in vitro [46], including transformed ones, [53], and to bind in vivo to endothelium of different microvascular beds including the murine myocardium [8, 27, 44, 54].

Lectins and their endogenous glycoligands are considered to be crucial in the transmission of molecular information during development of the heart [16]. Endothelium appears to express a common set of major glycoproteins in culture and in situ [48]. However, cell transformation may also lead to altered expression of cell surface lectin-binding properties [7]. The fact that MHEC5-T cells maintained the endothelium-specific glycoconjugates recognized by DBA and BSL I parallels the findings obtained with human endothelial cells, where the endothelium-specific UEA I lectin bound both normal and neoplastic endothelial cells [20, 29, 31, 47].

The tests for the presence of vimentin and cytokeratin were carried out to determine whether the MHEC5-T cells had acquired new properties consistent with their morphological transition to the epithelioid phenotype. Mesenchymal cells, including endothelial cells, typically express vimentin, whereas cytokeratin is generally considered to be specific for epithelial cells [13, 19]. However, in some cases, cytokeratin expression has been found in normal endothelia or in vascular tumours [22]. Only vimentin was detectable in the parental MHEC5 cell line, but cytokeratin as well as vimentin were present following transformation.

It is only recently that cultured tumour cells and transformed cells of endothelial origin have been characterized [18, 24, 33, 51, 52, 53, 55]. All of these cell lines have been found to induce tumours diagnosed as characteristic of haemangioma, haemangioendothelioma or haemangiosarcoma. The model of a vascular neoplasm presented by our study differs from those previously described in that it produces an unusual and rare but distinct vascular tumour in vivo. Epithelioid haemangioendotheliomas have a unique microscopic appearance characterized by the proliferation of epithelioid-like endothelial cells displaying remarkable pleomorphism. Epithelioid haemangioendotheliomas are considered to be borderline malignant tumours and display an atypical and heterogeneous growth pattern, thus often being misdiagnosed as carcinomas or sarcomas including Kaposi's sarcoma [56]. Epithelioid haemangioendotheliomas have been described in liver, bone, lung, brain and heart [10, 22, 26, 32].

Transitional stages of differentiation are frequently found during the development of neoplasms, including tumours of the heart. The cell line described in this report demonstrates that the in vitro transformation of myocardium-derived endothelial cells, evidenced by the loss of contact inhibition of growth, increased chemokinetic activity and altered surface adhesive properties, has led to a new stable phenotype characterized by endothelial – to – epithelioid transition. Although the concept of transformation of cell types from endothelium to epithelium has been proposed previously on theoretical grounds, the present report provides direct experimental evidence that heart endothelial cells can be modulated to an epithelioid phenotype. It appears significant that several of the phenotypic changes in clone MHEC5-T were presaged by low level expression in the untransformed and non-tumorigenic clone MHEC5. The study of MHEC5-T cells may help to elucidate regulatory mechanisms involved in endothelial cell activation, transition and transformation.

Acknowledgements The excellent technical support of Barbara Gilligan, Louis Kubai, Christine Neumüller and Gaby Rußmeier is gratefully acknowledged. We appreciate the assistance of Wanda Auerbach in bibliographic retrieval and editorial advice. We are grateful to Don Chandler, Bill Feeny and Barbara Ruppel for help in preparing the figures. We thank Edward Bersu for advice on karyotype analysis, Paul Kincade for furnishing antibodies to VCAM-1 and Daniel Malone for providing us with dilAcLDL.

References

1. Ades EW, Hierholzer JY, George V, Black J, Candal F (1992) Viral susceptibility of an immortalized human microvascular endothelial cell line. J Virol Methods 39:83–90
2. Auerbach R (1991) Vascular endothelial cell differentiation: organ-specificity and selective affinities as the basis for developing anti-cancer strategies. Int J Radiat Biol 60:1–10
3. Auerbach R (1991) Interactions between cancer cells and the endothelium. In: FW Orr, MR Buchanan, L Weiss (eds) The microcirculation in cancer metastasis. CRC Press, Boca Raton, pp 169–181
4. Auerbach W, Auerbach R (in press) Endothelial cell heterogeneity. Int Rev Cytol
5. Auerbach R, Lu WC, Pardon E, Gumkowski F, Kaminska G, Kaminski M (1987) Specific adhesion between murine tumor cells and capillary endothelium: an in vitro correlate of preferential metastasis in vivo. Cancer Res 47:1492–1496
6. Auerbach R, Plendl J, Kusha B (1992) Endothelial cell heterogeneity and differentiation. In: Maragoudakis ME (ed) Angiogenesis in health and disease. Plenum Press, New York, pp 55–62
7. Augustin-Voss HG, Smith CA, Lewis RM (1990) Phenotypic characterization of normal and neoplastic canine endothelial cells by lectin histochemistry. Vet Pathol 27:103–109
8. Bankston PW, Porter GA, Milici AJ, Palade GE (1991) Differential and specific labeling of epithelial and vascular endothelial cells of the rat lung by Lycopersicon esculentum and Griffonia simplicifolia I lectins. Eur J Cell Biol 54:187–195
9. Bautch VL, Toda S, Hassell JA, Hanahan D (1987) Endothelial cell tumors develop in transgenic mice carrying the polyoma virus middle T oncogene. Cell 51:529–553
10. Bernhards J, Aebert H, Maschek H, Ehrenheim CH, Werner M (1992) Epithelioides Hämangiom des Herzens. Pathologe 13: 39–44
11. Blood CH, Zetter BR (1990) Tumor interaction with the vasculature: angiogenesis and tumor metastasis. Biochim Biophys Acta 1032:89–118
12. Bollinger BK, Lasking WB, Knight CB (1994) Epithelioid hemangioendothelioma with multiple site involvement. Literature review and observations. Cancer 73:610–615
13. Chan JK, Frizzera G, Fletcher CD, Rosai J (1992) Primary vascular tumors of lymph nodes other than Kaposi's sarcoma. Analysis of 39 cases and delineation of two new entities. Am J Surg Pathol 6:335–350
14. Dubois NA, Kolpack LC, Wang R, Azizkhan RG, Bautch VL (1991) Isolation and characterization of an established endo-

thelial cell line from transgenic mouse hemangiomas. Exp Cell Res 196:302–313
15. Faught PR, Waller BF, Hull MT (1988) Spindle sarcoma of the heart in childhood: light microscopic, ultrastructural, and immunohistochemical evidence for smooth muscle, endothelial, and fibroblast differentiation. Pediatr Pathol 8:649–656
16. Frunchak YN, Martha GN, McFadden KD, Milos NC (1993) Alterations of heart development in Xenopus laevis by galactoside-binding lectin or its sugar hapten inhibitor. Anat Embryol (Berl) 187:299–316
17. Gimbrone MA Jr, Fareed GC (1976) Transformation of cultured human vascular endothelium by SV40 DNA. Cell 9: 685–693
18. Gorman SD, Hoffman E, Nichols WW, Cristofalo VJ (1984) Spontaneous transformation of a cloned cell line of normal diploid bovine vascular endothelial cells. In Vitro Cell Dev Biol 20:339–345
19. Gray MH, Rosenberg AE, Dickersin GR, Bhan AK (1990) Cytokeratin expression in epithelioid vascular neoplasms. Hum Pathol 21:212–217
20. Griffith DW, Stephenson TJ (1988) Lectin agglutinins: a comparative study of Lotus tetragonolobus and Ulex europaeus agglutinin I as markers of vascular endothelium in follicular thyroid carcinoma. Med Lab Sci 45:45–51
21. Gumkowski F, Kaminska G, Kaminski M, Morrissey LW, Auerbach R (1987) Heterogeneity of mouse vascular endothelium. In vitro studies of lymphatic, large blood vessel and microvascular endothelial cells. Blood Vessels 24:11–23
22. Haelst UJGM van, Pruszczynski M, Cate LN ten (1990) Ultrastructural and immunohistochemical study of epithelioid hemangioendothelioma of bone: coexpression of epithelial and endothelial markers. Ultrastruct Pathol 14:141–149
23. Hoch-Ligeti C, Stewart HL (1984) Cardiac tumors of mice. J Natl Cancer Inst 72:1449–1456
24. Hoover ML, Vetvicka V, Hoffpauir JM, Tamburro CH (1993) Human endothelial cell line from an angiosarcoma. In Vitro Cell Dev Biol 29A:199–202
25. Janigan DT, Husain A, Robinson NA (1986) Cardiac angiosarcomas. A review and a case report. Cancer 57:852–859
26. Kanavaros P, Lavergne A, Dellagi K, Elaerts J, Ouzan J, Piwnica A (1988) Hémangiome histiocytoide cardiaque. Etude histologique et immunohistochimique d'un cas. Ann Pathol 8: 228–233
27. Laitinen L (1987) Griffonia simplicifolia lectins bind specifically to endothelial cells and some epithelial cells in mouse tissues. Histochem J 19:225–234
28. Lam KI, Dickens P, Chan ACL (1993) Tumors of the heart. A 20-year experience with a review of 12,485 consecutive autopsies. Arch Pathol Lab Med 117:1027–1031
29. Little D, Said JW, Siegel RJ, Fealy M, Fishbein MC (1986) Endothelial markers in vascular neoplasms: an immunohistochemical study comparing Factor VIII-related antigen, blood group specific antigens, 6-keto-Pg alpha, and Ulex europaeus I lectin. J Pathol 149:89–95
30. Marchiano D, Fisher F, Hofstetter S (1993) Epithelioid hemangioendothelioma of the heart with distant metastases. A case report and literature review. J Cardiovasc Surg (Torino) 34: 529–533
31. Miettinen M, Holthöfer H, Lehto V, Miettinen A, Virtanen I (1983) Ulex europaeus I lectin as a marker for tumors derived from endothelial cells. Am J Clin Pathol 79:32–36
32. Miettinen M, Lindenmayer AE, Chaubal A (1994) Endothelial cell markers CD31, CD34, and BNH9 antibody to H- and Y-antigens. Evaluation of their specificity and sensitivity in the diagnosis of vascular tumors and comparison with von Willebrand factor. Mod Pathol 7:82–90
33. Miyake K, Medina K, Ishihara K, Kimoto M, Auerbach R, Kincade PW (1991) A VCAM-like adhesion molecule on murine bone marrow stromal cells mediates binding of lymphocyte precursors in culture. J Cell Biol 114:557–565
34. Murray AB (1987) Weibel-Palade bodies are not reliable ultrastructural markers for mouse endothelial cells. Lab Anim Sci 37:483–485
35. Nicolson GL (1988) Organ specificity of tumor metastasis: role of preferential adhesion, invasion, and growth of malignant cells at specific secondary sites. Cancer Metastasis Rev 7: 143–188
36. Obeso JL, Auerbach R (1984) A new microtechnique for quantitating cell movement in vitro using polystyrene bead monolayers. J Immunol Methods 79:141–152
37. Obeso J, Weber J, Auerbach R (1990) A hemangioendothelioma-derived cell line: Its use as a model for the study of endothelial cell biology. Lab Invest 63:259–269
38. Pitts OM, Powers JM, Hoffman PM (1983) Vascular neoplasms induced in rodent central nervous system by murine sarcoma viruses. Lab Invest 49:171–182
39. Plendl J (1985) Die spezifische Darstellbarkeit von Blutgefäßen der NMRI Maus während der Organogenesis mit Dolichos biflorus Agglutinin. Dissertation, University of Munich, Germany
40. Plendl J, Schmahl W (1988) Binding of Dolichos biflorus agglutinin, DBA, to endothelial cells during the embryonal period is restricted to the NMRI-mouse. In: Bog-Hansen TC, Van Driessche E (eds) Lectins, vol V. Walter de Gruyter and Company, Berlin, pp 507–514
41. Plendl J, Hartwell L, Auerbach R (1993) Organ-specific change in Dolichos biflorus lectin binding by myocardial endothelial cells during in vitro cultivation. In Vitro Cell Dev Biol 29A:25–31
42. Ponder BAJ, Wilkinson MM (1983) Organ-related differences in binding of Dolichos biflorus agglutinin to vascular endothelium. Dev Biol 96:535–541
43. Ponder BAJ, Festing MF, Wilkinson MM (1985) An allelic difference in binding of Dolichos biflorus lectin in inbred strains of mice. J Embrol Exp Morph 87:229–239
44. Porter GA, Palade GE, Milice AJ (1990) Differential binding of the lectins Griffonia simplicifolia I and Lycopersicon esculentum to microvascular endothelium: organ-specific localization and partial glycoprotein characterization. Eur J Cell Biol 51:85–95
45. Rutgers JF, Wieczorek R, Bonetti F, Kaplan KL, Posnett DN, Friedman-Kien AE, Knowles DM (1986) The expression of endothelial cell surface antigens by AIDS-associated Kaposi's sarcoma: Evidence for a vascular endothelial origin. Am J Pathol 12:493–499
46. Sahagun G, Moore SA, Fabry Z, Schelper RL, Hart MN (1989) Purification of murine endothelial cell cultures by flow cytometry using fluorescein-labelled Griffonia simplicifolia agglutinin. Am J Pathol 134:1227–1232
47. Schelper RL, Olson SP, Carroll TJ, Hart MN, Witters E (1986) Studies of the endothelial origin of cells in systemic angioendotheliomatosis and other vascular lesions of the brain and meninges using Ulex europaeus lectin stains. Clin Neuropathol 5:231–237
48. Schnitzer JE, Shen CPJ, Palade GE (1990) Lectin analysis of common glycoproteins detected on the surface of continuous microvascular endothelium in situ and in culture: identification of sialoglycoproteins. Eur J Cell Biol 52:241–251
49. Srebernik N, Appleby EC (1991) Breed prevalence and sites of hemangioma and hemangiosarcoma in dogs. Vet Rec 129: 408–409
50. Takagi M (1984) Ultrastructural and immunohistochemical characteristics of cardiac myxoma. Acta Pathol Jpn 34: 1099–1114
51. Takahashi K, Sawasaki Y (1992) Rare spontaneous transformed human endothelial cell line provides useful research tool. In Vitro Cell Dev Biol 28A:380–382
52. Takahashi K, Sawasaki Y, Hata JI, Mukai K, Goto T (1990) Spontaneous transformation and immortalization of human endothelial cells. In Vitro Cell Dev Biol 25:265–274

53. Toda KI, Tsujioka K, Maruguchi Y, Ishii K, Miyachi Y, Kuribayashi K, Imamura S (1990) Establishment and characterization of a tumorigenic murine vascular endothelial cell line (F-2). Cancer Res 50:5526–5530
54. Tontsch U, Bauer HC (1989) Isolation, characterization, and long-term cultivation of porcine and murine cerebral capillary endothelial cells. Microvasc Res 37:148–161
55. Tsujioka K, Toda KI, Maruguchi Y, Miyachi Y, Imamura S (1992) Morphological characterization of hemangiomatous tumors derived from a novel murine vascular endothelial cell line (F-2). J Dermatol 18:506–516
56. Weiss SW, Enzinger FM (1982) Epithelioid hemangioendothelioma: A vascular tumor often mistaken for a carcinoma. Cancer 50:970–981
57. Williams RJLL, Robertson D, Davies AJS (1989) Identification of vascular endothelial cells in murine omentum using the lectin Dolichos biflorus agglutinin: possible applications in the study of angiogenesis. Histochem J 21:271–278
58. Yuen PH, Matherne CM, Molinari-Storey LM (1991) SV7, a molecular clone of Moloney murine sarcoma virus 349, transforms vascular endothelial cells. Am J Pathol 139:1449–1461
59. Zetter BR (1989) Endothelial heterogeneity: influence of vessel size, organ localization and species specificity on the properties of cultured endothelial cells. In: Ryan US (ed) Endothelial cells, vol 2. CRC Press, Boca Raton, pp 63–79