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Published in:
Angiogenesis

DOI:
10.1023/A:1012259529167

Angiogenesis **4**: 91–102, 2001.

Review

Endothelium *in vitro*: A review of human vascular endothelial cell lines for blood vessel-related research

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Received 16 January 2001; accepted in revised form 16 May 2001

Key words: cell line, endothelium, immortalized, *in vitro*, review

Abstract

Endothelial cells (EC) are currently used as *in vitro* model systems for various physiological and pathological processes, especially in angiogenesis research. Primary EC have a limited lifespan and display characteristics that differ from batch to batch due to their multidonor origin. In recent years many groups have established EC lines. This Review gives an overview of the advantages and disadvantages of currently available vascular EC lines. Its aim is to help the investigator to decide which cell line matches his or her research goal best. Truly immortalized cell lines are generally better characterized and more stable in their endothelial traits than EC that were given an extended life span. Presently the best characterized macro- and micro-vascular EC lines are EA.hy926 and HMEC-1, respectively.

Abbreviations: ACE – angiotensin converting enzyme; acLDL – acetylated low density lipoprotein; ADP – adenosine-diphosphate; AECA – anti-endothelial cell antibodies; AIDS – acquired immune deficiency syndrome; bFGF – basic fibroblast growth factor; CD – cluster of differentiation; EC – endothelial cell; ECE – endothelin-converting enzyme; ECGF – endothelial cell growth factor; ECGS – endothelial cell growth solution as used in Ref. [1]; ECM – extracellular matrix; EDRF – endothelium-derived relaxing factor; ET – enothelin; FCS – fetal calf serum; FVIIIrAg – Factor VIII-related antigen (= von Willebrand factor); GM-CSF – granulocyte-macropage colony-stimulating factor; HAEC – human aortic endothelial cell; HIVEC – human iliac vein endothelial cell; HMEC – human (dermal) microvascular endothelial cell; HPEC – human placental endothelial cell; HUVEC – human umbilical vein endothelial cell; ICAM-1 – intercellular adhesion molecule 1; IGF-1 – insulin-like growth factor 1; IFN-γ – interferon γ; Il-1 – interleukin-1; KS – Kaposi's sarcoma; LFA-1 – leukocyte function antigen-1; LPS – lipopolisaccaride; mAb – monoclonal antibody; MMP – matrix metalloproteinase; NF-κB – nuclear factor κB; NO – nitric oxide; PAF – platelet-activating factor; PAI-1 – plasminogen activator inhibitor-1; PBMC – peripheral blood mononuclear cells; PDL – population doublings; PECAM-1 – platelet endothelial cell adhesion molecule 1 (= CD31); PGl – prostacyclin; PMA – phorbol-12-myristate-13-acetate; SAINT – synthetic amphiphiles interactive; SMC – smooth muscle cell; SV40 – simian vacuolating virus 40; TF – tissue factor; TFPI – tissue factor pathway inhibitor; TM – thrombomodulin; TNF-α – tumor necrosis factor α; tPA – tissue-type plasminogen activator; UEA – *Ulex europaeus* lectin agglutinin I; uPA – urokinase-type plasminogen activator; VCAM-1 – vascular cell adhesion molecule 1; VEGF – vascular endothelial growth factor; WP-bodies – Weibel–Palade-bodies

Introduction

Blood vessels belong to the largest organs of our body. The total vascular surface of an adult has been estimated to be 7 m^2 [2], with a total weight of endothelial cells (EC) in an adult of 720 g [3].

All blood vessels, from the largest arteries and veins to the smallest postcapillary venules, are lined with EC. The functions of these cells are multiple and can vary considerably depending on the localization and size of their corresponding blood vessels [4]. EC do not form a passive barrier; they can actively transport small molecules, macromolecules and hormones such as insulin, and degrade lipoprotein particles (reviewed in [5]). EC furthermore play major roles in blood pressure regulation, blood coagulation and fibrinolysis, adhesion and transmigration of inflammatory cells on their way out of the vessel into the target tissue, and angiogenesis, the

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formation of new blood vessels. Additionally, they may represent a target for autoantibodies in autoimmune disorders such as systemic lupus erythematosus, rheumatoid arthritis and Kawasaki's disease (reviewed by [2]). Aberrant EC function can therefore lead to pathological processes as diverse as atherogenesis [5], bleeding disorders (reviewed by [6]), autoimmune disorders, graft rejection [2] or even embryonic lethality [7].

The growing interest in the role of endothelium in physiological and pathological conditions has led to an increased demand for representative *in vitro* model systems for vascular tone, blood coagulation, angiogenesis, lymphocyte adhesion and transmigration as well as signalling and transport routes across blood- and lymphatic-vessel walls.

Endothelium as an in vitro *model*

A major part of our knowledge on EC functions comes from *in vitro* experiments with HUVEC (human umbilical vein endothelial cells [8]). HUVEC, as well as other primary EC have an average life span of 10 serial passages and can be kept in culture up to 5 months [8]. Thereafter the cells enter a stage referred to as senescence, where they stop proliferating, tend to form giant, multinucleated cells and finally die. This implies that no long-term *in vitro* experiments can be performed with HUVEC. The isolation of primary HUVEC is laborious and experimental results obtained with different HUVEC isolates cannot easily be compared to each other because of their different donor origin. Watson et al. [9] reported for example, that the response to Il-8-stimulation is different between several commercially available HUVEC (and their growth media and conditions) and 'home isolated' and cultured HUVEC. The behavior of EC also strongly differs depending on their vascular origin. Human placental endothelial cells (HPEC [10]) show a different morphology than HUVEC, the cells are more elongated, form networks in sparse cultures and grow in expanded spiral structures of elongated cells at confluence. They even form tubular structures on the surface of the original monolayer.

The first attempts to keep EC in long-term culture used tumor-conditioned medium, gelatin-coated plates and EC enrichment in the primary culture [11]. Still this approach did not overcome the problem of the heterogeneity of EC donors. Furthermore primary EC tend to loose their primary characteristics and responsiveness to various stimuli beyond passage 6 (own observation). For a recent review about isolation and cultivation of primary EC, see [3].

The need for standardized experimental conditions and reproducible results has increased the demand for immortalized, well-characterized EC lines stably presenting endothelial properties. However, the search for the ideal immortalized cell line showing all the properties of the primary cells, but no traits of tumor cells is a contradiction in itself. The achievement of immortality is already an indication of non-physiologic if not tumorigenic conversion. Looking for an EC line, the goal is to find the cell line presenting most of the desired primary characteristics with as few as possible tumor cell traits.

Typical endothelial characteristics can be divided into phenotype and function:

A. Phenotype
- The presence of Weibel–Palade-bodies (WP-bodies) [12]. These are large rod-shaped organelles that are specific for EC. WP-bodies store large amounts of von Willebrand Factor (vWF) also known as Factor VIII-related antigen (FVIIIrAg) that can quickly be released upon activation of the cells [13].
- vWF-secretion. This can be constitutive or regulated and also occurs in the absence of WP-bodies. vWF is a large adhesive glycoprotein synthesized in EC and megakaryocytes. In the blood it serves as a stabilizing carrier for Factor VIII with which it circulates as a complex (reviewed in [14]).
- ICAM, VCAM and E-selectin-expression which are upregulated upon activation [15].
- VE-cadherin expression in the cellular junctions [16].

B. Function
- Binding of the *Ulex europaeus* lectin agglutinin I (UEA) [17].
- Uptake of acetylated low density lipoprotein (acLDL) [18].
- Angiotensin-converting enzyme (ACE)-activity [19].

Characteristics one tries to avoid when looking for an EC line are those of degenerated cells or tumor cells as for example an aneuploid or unstable karyotype, growth in soft agar and tumorigenicity in nude mice.

Looking for a cell line with which to start experimental series, one has to determine which properties are crucial to validate the experimental setting and which ones are desirable to simplify experimental procedures. For example an interest in protein production and purification might lead to the choice of a cell line growing on serum or protein-free medium. The examination of the mechanisms of cell senescence and immortality might result in the use of a cell line with extended but limited life span as well as a stable karyotype or in a telomerase-expressing cell line.

Scientists looking for blood–endothelium interactions would prefer cell lines that express specific adhesion molecules or members of the coagulation cascade like tissue factor (TF) or tissue-type plasminogen activator (tPA) and/or show lymphocyte adhesion.

Desirable traits of a cell line for angiogenesis research would be tube formation on Matrigel, contact inhibition and anchorage dependence.

There are also a number of EC lines with specific properties of the organs or pathologic conditions of the patients they have been isolated from. Researchers interested in Kaposi's sarcoma (KS) would be choosing a KS-derived cell line, whereas interest in the blood–brain barrier would lead to a cerebromicrovascular cell line.

A number of human EC lines have been established, characterized and used for various research purposes. In this Review we summarize the current knowledge about these cell lines and the purposes they have been used for. We have focused on human vascular in contrast to lymphatic EC lines, because most of the knowledge available so far concerns vascular endothelium and more established vascular cell lines have been reported to date. Animal cell lines were not taken into account as human EC presumably reflect the human *in situ* situation incompletely but better than EC from any other organism.

The purpose of this review is to facilitate the choice of cell lines and models by analyzing the merits and disadvantages of various systems reported.

In general there are two types of EC lines: (a) EC with a prolonged life span that get over senescence but die in crisis (Table 1) and (b) immortalized EC that survived crisis and are stable in their morphology and antigen expression (Table 2).

We have emphasized on truly immortalized cell lines as for most applications immortalized cell lines are more practical than primary cells which have been given an extended life span by transfection, infection or fusion with an immortal cell. However, especially for studies regarding the senescence of endothelium, cell lines that are not immortalized might be useful. Many of those cell lines were produced before immortalized cell lines became available. None of them has been widely used in blood vessel-related research. There is also often less information as to the properties of the cells. We have compiled the references on these specific cells but will not discuss each cell line in detail.

The established, immortalized cell lines can be further subdivided into 'large-vessel endothelium', 'small-vessel endothelium' and finally 'non-vessel endothelium'. The last group referring to bone marrow EC, or pathologic EC isolated from patients lesions or ascites.

Endothelial cell lines with prolonged life span

As early as 1976 Gimbrone and Fareed transfected HUVEC yielding the cell lines SVHEC-A, -B, -C, -D and -F [20]. In 1988 Ide et al. [21] followed with SV-HUVEC obtained by SV40 virus infection of HUVEC. The same year Salahuddin et al. [22] isolated endothelium of an AIDS-Kaposi sarcoma (AIDS-KS) patient leading to the cell lines AIDS-KS1, -2, -3, -4, -5 and AIDS-KS6. In 1989 and 1991 two additional reports about AIDS-KS cell lines followed: SV-KSC [23] and KS1, KS2, KS3 and KS4 [24], the former being transfected with SV40 DNA the latter was unaltered. They were succeeded by SV-2 [25] and SGHEC-7 [26], two unnamed cell lines reported by Hohenwarter et al. [27, 28] and EC-pSV1 [29]. The latter of Hohenwarter's cell lines was established by fusion of HUVEC with the human osteosarcoma cell line 134B, the others by transfection of HUVEC with SV40 genes. Further the establishment of five cell lines, each growing in different defined growth media ranging from rich media to medium without any growth factors added (ESV108/2010-GF/2010ECGS/233 and ESV2010INS/EGF) was reported [1].

One of the few microvascular non-immortalized cell lines reported is HPEC-A1 [30]. It was generated by transformation of placental EC (HPEC, [10]) with SV40 early region genes in 1997. In brief, the most appropriate models for non-immortalized EC with extended life span seem to be SV-2 [25], the two unnamed cell lines of Hohenwarter et al. [27, 31] and the microvascular cell line HPEC-A1 [30].

However, there are several immortalized cell lines that are better characterized and present the advantages of immortality: a stable karyotype and phenotype. Based on this, these prolonged life span EC lines are likely to be most useful in research that explicitly requires non-immortalized cells.

Immortalized cell lines

Large vessel endothelium

One of the most frequently used and best characterized permanent human vascular EC lines is EA.hy926 [14]. The cell line was generated in 1983 by fusion of HUVEC with the human lung carcinoma cell line A549 [32]. The resulting hybrids had more chromosomes than their progenitors and carried a marker chromosome from the A549 line. The cells are contact inhibited in growth, show reduced growth factor requirements, express vWF [14] and upregulate ICAM-1, VCAM-1 and E-selectin expression upon stimulation with TNF-α but not with Il-4 and IFN-γ [33]. EA.hy926 cells also express tPA, PAI-1, TF and TM (reviewed by [34]). Pronectin (= CD59)-expression was upregulated by the protein kinase C inducer phorbol-12-myristate-13 acetate (PMA), the calcium ionophore A23187 and the protein kinase A inducer dibutyryl-cyclic adenosine monophosphatase [35]. EA.hy926 has been used for adhesion assays with several human leukocyte cell lines [33], human peripheral blood mononuclear cells (PBMC) in the presence of blocking monoclonal antibodies (mAbs) [36], unstimulated human neutrophils in the presence of dexamethasone [37] and murine T cells [38]. Walkden et al. [39] used EA.hy926 to investigate the membrane anchorage of endothelin-converting enzyme (ECE).

Another presumed permanent human EC line is ECV304 [40], reported to have emerged from a spontaneous transformation event in routinely cultured HUVEC in 1985 and first described in 1990. Lately, some doubts as to the endothelial origin of ECV304 have been voiced, the cell–cell contacts suggesting a (partly) epithelial origin [41]. Finally, Brown et al. [42] showed genetical identity of ECV304 to the human bladder cancer-derived epithelial cell line T24/83. ECV304 is therefore no longer to be considered of endothelial

Table 1. EC with an extended life span.

	Parental cells	Cell line	Transformation SV40	Karyotype	Contact inhibition	Ancho-rage	Reduced serum	Matrigel	Uptake acLDL	WP-bodies	Tumor	Soft agar	vWF/ FVIIIrAg	ACE	UEA	ICAM, VCAM, E-sel	Reference
Large vessels	HUVEC	SVHEC-A, B.. -F	+	Aneuploid	−	−	+	?	?	−	?	?	−	±	?	?	[20]
	HUVEC	SV-HUVEC	+	45	+	?	+	?	?	?	±	−	+	?	+	?	[21]
	HUVEC	SV-2	+	±Diploid	?	+	−	?	?	?	?	−	+	?	?	?	[25]
	HUVEC	SGHEC-7	+	42 Instable	+	−	+	?	?	?	?	?	(+)	?	?	TNF, II1	[26]
	HUVEC	?	+	85	+	+	+	?	?	+	?	?	+	?	?	?	[27]
	HUVEC+ 143B	EC∗ (EC∗143B)	Fusion	102	?	?	+	?	?	?	?	?	+	?	?	?	[28]
	HUVEC	EC-pSV1	+	?	−	?	+	?	?	−	?	−	−	+	?	ICAM	[29]
	HUVEC	ESVSF108	+	>50% Diploid	?	?	+	?	?	?	?	?	+	?	?	?	[1]
	HUVEC	ESV233	+	>50% Triploid	?	?	+	?	?	?	?	?	?	?	?	?	[1]
	HUVEC	ESV2010 ECGS	+	?	?	?	+	?	?	?	?	?	?	?	?	?	[1]
	HUVEC	ESV2010 INS/EGF	+	?	?	?	+	?	?	?	?	?	?	?	?	?	[1]
	HUVEC	ESV2010 -GF	+	?	?	?	+	?	?	?	?	?	?	?	?	?	[1]
Small vessels	HPEC	HPEC-A1	+	?	(+)	?	?	?	+	?	?	?	+	?	+	?	[30]
Patient material	Kaposi sarcoma	AIDS-KS1 -6	−	46XY	?	?	?	?	+	−	−	?	−	−	+	?	[22]
	Kaposi sarcoma	SV-KSC	+	?	−	−	+	?	?	?	−	+	?	?	?		[23]
	Kaposi sarcoma	KS1, ...- KS4	−	?	+(−)	+	+	−	?	?	?	?	+	?	+	?	[24]

Transformation SV40: transformation with simian virus 40 genes (+) or other methods specified.
Karyotype: mean number of chromosomes.
Contact inhibition: cells grow in monolayers.
Anchorage: cells do not grow in suspension.
Reduced serum: the cells require 10% or less serum and/or have reduced growth factor requirements.
Matrigel: cells form tubular structures on Matrigel.
Cell adhesion: adhesion of peripheral blood cells to a cell monolayer.
Uptake acLDL: incorporation of acidic low denisty lipoprotein.
WP bodies: Weibel–Palade-bodies.
Tumor: cells are tumorigenic in nude mice.
Soft Agar: cells form clonies in soft agar.
vWF/FVIIIrAg: von Willebrand Factor (= Factor VIII-related antigen) expression.
ACE: Agiotensin converting enzyme activity.
UEA: *Ulex europeaus* lectin agglutinin I binding.
ICAM, VCAM, E-sel: the cells show expression of ICAM-1, VCAM-1 and E-selectin or can be induced to do so by specified stimulators.

Table 2. Immortalized EC lines.

	Parental cells	Cell line	Transformation SV40	Karyotype	Contact inhibition	Anchorage	Reduced serum	Matrigel	Cell adhesion	Uptake acLDL	WP-bodies	Tumor	Soft agar	vWF/ FVIIIr Ag	ACE	UEA	ICAM, VCAM, E-sel	tPA, PAI	TF, TM, TFPI	Reference
Large vessels	HUVEC+ A549	EA.hy 926	Fusion	80	+	?	+	?	Several Types		?	?	?	+	?	?	TNF	++	++−	[14]
	HUVEC	HEC-KSV	KSV	Dioploid	+(−)	?	−+	?	?	?	+	~	−	+	?	?	?	?	?	[47]
	HUVEC	HEC-MSV	MSV	Dioploid	+(−)	?	−+	?	?	?	−	~	−	+	?	?	?	?	?	[47]
	HUVEC	IVEC	+	48	+	−	+	?	?	+	+	?	?	+	+	+	Il1-ß	?	?	[48]
	HAEC	SE-1	+	?	+	?	+	?	?	?	−	−	−	+ (Weak)	−	+	?	?	?	[50]
	HUVEC	SV-3T	+	Aneuploid	?	+	−	?	?	?	?	?	+	−	?	?	?	++	?	[25]
	HUVEC	C11STH	Spontaneous	Diplo+ trisomy 8, 11	+−	?	−	+	Neutrophils	?	?	−	?	+	?	?	TNF	?	?	[52]
	HUVEC	EC-RF24	HPV	Diploid	+	+	−	?	Neutrophils	?	+	?	?	+	?	?	+	?	+	[53]
	HUVEC	CEHI	+	?	+	?	?	?	?	+	+	−	?	+	+	+	?	++	?	[54]
	HUVEC+ A549	A1g, C26a, C49b, C50b, C50c, C50d, C98f	Fusion & subcloning	?	?	?	?	?	?	?	?	?	?	−	?	?	+	+	?	[55]
	EA.hy926	CJE-hy3	Subcloning	?	?	?	?	?	?	?	?	?	?	−	?	?	+	++	?	[55]
	HUVEC	EVLC2	+	Unstable	+	?	+	?	?	?	?	?	?	−	?	?	?	++	+++	[34]
	HUVEC	hTERT-HUVEC	Adenovirus	Aneuploid	+	+	?	+	?	+	?	?	−	+	?	?	+	?	?	[56]
	HAEC	hTERT-HAEC	Adenovirus	Aneuploid	+	+	?	+	?	+	?	?	−	+	?	?	+	?	?	[56]
	HCEC	hTERT-HCEC	Adenovirus	Aneuploid	+	+	?	+	?	+	?	?	−	+	?	?	+	?	?	[56]
	HSVEC	hTERT-HSVEC	Adenovirus	Aneuploid	+	+	?	+	?	+	?	?	−	+	?	?	+	?	?	[56]
Small vessels	HMEC	HMEC-1	+	Mean diploid	+	+	++	+	Lymphocytes	+	?	?	−	+	?	+	+	++	++−	[63]
	HCEC	SV-HCEC	+	Hyper-diploid	+	+	−	+	?	+	?	?	−	+	?	?	?	?	?	[64]
	Fetal SEC	iSEC	Polyoma virus	?	+	+	?	?	?	?	−	?	?	+	?	?	?	?	?	[65]
	HMEC	hTERT-HDMEC	Adenovirus	Diploid	+	+	?	+	?	+	?	?	−	+	?	?	+	?	?	[56]
Bone marrow	BMEC	BMEC-1	+	?	+	?	-+	+	CD34+ cells	+	?	?	?	+	?	+	+	?	?	[76]
	BMEC	TrHBMEC	+	50	+	?	+	+	CD34+ cells	+	+	?	?	+	?	+	TNF	?	?	[77]

Table 2. Continued.

	Parental cells	Cell line	Transformation SV40	Karyotype	Contact inhibition	Anchorage	Reduced serum	Matrigel	Cell adhesion	Uptake acLDL	WP-bodies	Tumor	soft agar	vWF/FVIIIrAg	ACE	UEA	ICAM, VCAM, E-sel	tPA, PAI	TF, TM, TFPI	Reference
	BMEC	HBME-1	+	Diplo + hyper-triplod	+	+	-	+	Tumor cells	+	+	?	?	+	?	+	+	?	?	[78]
Patient material	Adenosarcoma	SK-HEP	–	62	+–	–	+	+	?	?	+	+	?	+	?	–	E-sel	?	?	[79]
	Adenosarcoma	HAEND	–	?	–	–	+	?	?	?	?	+	?	+	?	?	ICAM	?	?	[80]

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Karyotype: mean number of chromosomes.
Contact inhibition: cells grow in monolayers.
Anchorage: cells do not grow in suspension.
Reduced serum: the cells require 10% or less serum and/or have reduced growth factor requirements.
Matrigel: cells form tubular structures on Matrigel.
Cell adhesion: adhesion of peripheral blood cells to a cell monolayer.
Uptake acLDL: incorporation of acidic low denisty lipoprotein.
WP bodies: Weibel–Palade-bodies.
Tumor: cells are tumorigenic in nude mice.
Soft Agar: cells form clonies in soft agar.
vWF/FVIIIrAg: von Willebrand Factor (= Factor VIII-related antigen) expression.
ACE: Agiotensin converting enzyme activity.
UEA: *Ulex europeaus* lectin agglutinin I binding.
ICAM, VCAM, E-sel: the cells show expression of ICAM-1, VCAM-1 and E-selectin or can be induced to do so by specified stimulators.
tPA, PAI: tissue-type plasminogen activator, plasminogen activator inhibitor.
TF, TM, TFPI: tissue factor, thrombomodulin, tissue factor pathway inhibitor.
The cell lines ECV304 [40], t-HUE2 and t-HUE4 [43] were left out because recent data [42] have proven them to be of non-endothelial origin.

origin. The same accounts for the two sublines t-HUE2 and t-HUE4 of ECV304 reported by Kobayashi et al. [43]. For reviews about ECV304, see [44–46].

Faller et al. [47] reported the establishment of two stable human endothelial cell lines by murine sarcoma virus pseudotypes. HEC-KSV and HEC-MSV (KSV – Kirsten sarcoma virus; MSV – Moloney sarcoma virus). These cell lines are karyotypically, morphologically and phenotypically similar to primary HUVEC. The cells have a diploid female karyotype and are – as opposed to primary cells – serum but not specific growth factor dependent. HEC-KSV contain WP-bodies and release intracellular vWF upon PMA-stimulation, whereas HEC-MSV only show vWF expression but no WP-bodies. They also synthesize GM-CSF (granulocyte-macrophage colony stimulating factor), bind human lymphocytes via an LFA-1-mediated (leukocyte function antigen 1) mechanism and can be induced to express EC-specific acute activation antigen recognized by mAb H4/18. Tumorigenicity assays with viable and lethally irradiated HEC-KSV cells yielded tumors after long lag periods. The tumors seemed to be sarcomas of murine origin, similar to those obtained by inoculation with murine sarcoma viruses. No virus particles were detected but genomic transcripts of helper and sarcoma virus were, implying the shedding of minimal amounts of infectious virus particles by the HEC-KSV and HEC-MSV cell lines.

Schwartz et al. [48] generated two EC clones by transfection with SV40 genes, one of which was later named IVEC for immortalized venous EC [49]. IVEC bind UEA, take up acLDL, synthesize Factor VIII and FVIIIrAg (= vWF) and show activated expression of E-selectin, ICAM-1 and VCAM-1 upon stimulation with Il-1β, similar to HUVEC. Their ACE-activity is lower than in primary cells and can be completely blocked by the specific ACE-inhibitor Enaprilat. Prostacyclin secretion is induced by PMA or thrombin [48, 49].

Sasaguri et al. [50] isolated aortic EC (HAEC) from a 52-year-old male who died of lung cancer and treated them with SV40 virus, yielding the cell line SE-1 [51]. The SE-1 cells grew in monolayers, were weakly positive for vWF but did not contain WP-bodies. They further showed UEA-binding, had a well-developed fibronectin network, expressed TF, TM and TFPI and were not tumorigenic [50] when injected in mice. They have further been used as a model system for the role of blood pressure on endothelial cell proliferation and the production of MMP-1 [51].

Besides generating the SV2 cell line with an extended life span, Iijima et al. [25] also isolated an immortalized clone, SV-3T during the same procedure. Atypical for transformed cells, the cell line is anchorage dependent and does not have reduced growth factor requirements. Still, it can grow in soft agar. In opposition to SV2, SV-3T does not express vWF. SV-2 and SV-3T produce more tPA than primary EC and PAI-1 at the same level as HUVEC.

A spontaneous transformation event in a HUVEC culture was reported by Cockerill et al. [52]. The resulting cell line, termed C11STH, had trisomies of chromosomes 8 and 11. The expression of vWF, P-selectin, TNF-α-receptor and scavanger receptor was similar to that in HUVEC. TNF-α treatment induced expression of E-selectin, VCAM-1 and ICAM-1. C11STH is not tumorigenic but can form tubes on Matrigel and shows neutrophil adhesion *in vitro*.

Fontijn et al. [53] immortalized HUVEC by integration of the genes E6/E7 of human papilloma virus, yielding the cell lines EC-RF7 and EC-RF24, the authors mainly describing the characteristics of the latter. The EC-RF24 cells are diploid, grow in a polar mode, are contact inhibited and anchorage dependent. Their expression levels of endothelium-specific markers as vWF, surface-bound endoglin, PECAM-1, E-selectin, VCAM-1 and ICAM-1 as well as of non-endothelial-specific markers CD9, CD13, CD14, CD29, CD36, CD40, CD51 and CD55 are identical to those in HUVEC. EC-RF24 cells show a similar neutrophil transmigration rate to HUVEC, have WP-bodies and show constitutive PAI-1 synthesis and inducible TF expression and deposition of both in the subendothelial matrix.

Moldovan et al. [54] immortalized HUVEC in order to study the endothelin-converting enzyme ECE as Walkden and Turner [39] did with EA.hy926. The resulting cell line CEHI (cellules endotheliales humaines immortalisées) expressed vWF, tPA, PAI-1, ACE and Vimentin, took up acLDL, bound UEA and proliferated twice as fast as HUVEC. The cells further secreted Il-1β and Il-6 in a constitutive and lipopolysaccharide (LPS)-induced manner and were not tumorigenic in nude mice. The authors also showed arginine transport into CEHI cells, being a key step in endothelium-derived relaxing factor (EDRF) production (= NO-synthase activity).

The aim of Tonquèze et al. [55] was to obtain sets of endothelial clones with individual antigens to define anti-EC antibodies (AECA) patterns in the hope that some would express disease-specific autoantigens. They therefore fused HUVEC with A549/8 [32] in a similar way as performed to establish the cell line EA.hy926 [14]. The resulting two clones Hy1 and Hy2 were subcloned leading to one subclone of the former, A1g, and six subclones of the latter, C26a, C49b, C50b, C50c, C50d and C98f. The authors also subcloned the cell line EA.hy926 yielding the clone CJE-hy3. All eight clones were positive for HLA class I and II, TNF-α-receptor, GMP-140, E-selectin, ICAM-1, VCAM-1, PECAM-1, LFA-3, the β-chain of VLA, tPA and vWF, however the expression levels varied.

Van Leeuwen et al. [34] reported the generation of the immortalized cell lines EVLB3 and EVLC2 from HUVEC and EVLK1 and EVLK2 from human iliac vein EC. Upon gene analysis, the four cell lines were shown to be derived from one single HUVEC-derived clone and therefore were renamed to EVLC2 (van Leeuwen et al., unpublished data). The cell line shows the typical

cobblestone morphology and expresses tPA, PAI-1, TF, TM and TFPI but is vWF-negative [34].

The interest in vessel ageing and apoptosis in EC has lead to the transformation of an array of primary EC from different vascular origins with the human telomerase reverse transcriptase (hTERT)-gene, being the catalytic component of telomerase [56]. HUVEC, HAEC (human aortic EC), HCAEC (human coronary artery EC), HSVEC (human saphenous vein EC) and HDMEC (human dermal microvascular EC, further described in the 'small-vessel' section) were immortalized either by transfection or by retroviral transduction leading to hTERT-positive clones. The TRF-lengths (terminal restriction fragment length) of the transformed cells first decreased, subsequently stabilizing at a low level (2–5 kbp). The cells showed contact-inhibited growth in cobblestone monolayers, vWF- and PECAM-1-expression, were positive for ICAM-1, VCAM-1 and E-selectin, could form tubes on Matrigel an collagen, took up acLDL and did not grow in soft agar. The transformation did not alter the genomic status of the EC. In contrast to microvascular EC, all studied types of large vessel parental EC showed 40% of aneuploidy at low passage numbers and 100% aneuploidy at high passage numbers, the corresponding hTERT cell lines also being 100% aneuploid. Interestingly, the hTERT-HAEC line was less susceptible to apoptotic induction than the parental control EC or control-transfected cells, the other large vessel cell lines had not yet been tested for apoptosis. The authors also showed that primary EC at low passage numbers and approaching senescence are less susceptible to apoptosis induction than primary EC around PDL25. Along with the fact that the four described macrovascular cell lines still present many traits of primary EC, the decreased apoptosis-susceptibility points at a primary phenotype of these cells.

Small-vessel endothelium

As the knowledge about endothelium increased, it became apparent that EC in large vessels have different functions from those in the microvasculature and that these differences are reflected in different phenotypical characteristics. Microvascular EC have been isolated from the human placenta [10], neonatal foreskins [57–59], the adult human dermis [60, 61], adipose tissue [62] and other microvascular environments. Yet, only a few immortalized microvascular endothelial cell lines have been established so far, by transfection with large T antigens of SV40 (HMEC-1 [63], SV-HCEC [64]), polyoma virus, (iSEC [65]) or hTERT-retrovirus (hTERT-HDMEC [56]).

Immortalized fetal sinusoidal liver cells, (iSEC) [65] show diffuse cytoplasmic vWF but no WP-bodies, a feature that may be characteristic for fetal endothelium. iSEC could not be cloned as they did not proliferate below 5×10^3 cells/ml. The cells synthesize the extracellular matrix components collagen IV and fibrinogen but not laminin or enactin. They further express cytokeratins 7, 8 and 18 and vimentin, indicating a mesenchymal origin. iSEC are able to extend the life span of cocultured primary hepatocytes through cell–cell contacts.

Ades et al. [63] established a human EC line from human dermal microvascular ECs (HMEC). The resulting cell line CDC/EU.HMEC-1 (commonly termed HMEC-1) can grow in the absence of human serum and to densities 3–7 times higher than primary microvascular EC. However, the cells show morphological, phenotypical and functional characteristics of human microvascular EC. They have a cobblestone morphology, secrete vWF, show uptake of acLDL, tube formation on Matrigel and specific binding of lymphocytes in adhesion assays [63]. The cells can either constitutively express a number of integrins, ICAM-1, VCAM-1 and E-selectin [66] as well as MHC class II, CD31, CD36, CD44, tPA, PAI-1, TF, TM and epitopes recognized by the mAbs EN4 and PAL-E [63] or can be induced to do so. HMEC-1 showed linear uptake of titrated cholesterol and was used in an anticholesterol drug assay [67]. The effect of the alkylphospholipid ET-18-OCH3 on HMEC-1 tube-formation, cell adhesion molecule expression and cell junction integrity was also investigated [68]. The cell line was further used to seed vascular grafts [69] and coronary stents [70]. Elevated glucose-concentration inhibited HMEC-1 proliferation, making it a model for diabetic microangiopathy [71]. HMEC-1 has also been used to study cytoskeletal changes in endothelial permeability as occurring during inflammatory responses. Histamin induced the formation of intercellular gaps in a reversible time- and concentration-dependent manner [72].

Besides establishing four macrovascular hTERT-positive cell lines Yang et al. [56] also established hTERT-HDMEC, presenting all the primary endothelial features described for the macrovascular lines but having the additional advantage of a stable, male, diploid karyotype. The cells were also less susceptible to apoptotic induction than the parental controls or control-transfected cells (just as hTERT-HAEC).

Muruganandam et al. [64] isolated and immortalized cerebromicrovascular EC (HCEC), generating SV-HCEC. The cells express vWF and PAs, take up acLDL, form tubes on Matrigel and are anchorage and serum dependent as primary HCEC, but do not grow on soft agar. SV-HCEC is further able to bind lectins, express transferrin receptor and exert transferrin receptor-mediated endocytosis. Blood–brain barrier specific enzymes as alkaline phosphatase and γ-glutamyl transpeptidase have high enzymatic activities in SV-HCEC. The diffusion of sucrose through the cell monolayer, being a measure for monolayer leakiness, was equal to or lower than through control microvasculature. The transendothelial electric resistance (TER), an indicator for tightness and/or density of junctional complexes, was higher than in control lung microvasculature and could be increased by conditioned media

from human fetal astrocytes. These properties make SV-HCEC a good *in vitro* model for the blood–brain barrier [64].

Non-vessel endothelium

Bone marrow endothelium

The human bone marrow contains ECs and their precursors. When bone marrow cells are mobilized in response to certain drugs and isolated from peripheral blood, bone marrow-associated EC can be isolated in parallel. Endothelial precursors have also been isolated from peripheral blood and can differentiate *in vitro* and *in vivo* to form EC [73–75] but no immortalized cell line has been reported so far. These EC cannot be categorized into small- and large-vessel derived endothelium but form a category by itself.

The three bone-marrow derived EC lines described in the literature showed quite similar features. They were all obtained by transfection with SV40 genes, the growth of the cell lines CDC/CU.BMEC-1 (commonly referred to as BMEC-1 [76]), TrHBMEC [77] as well as HBME-1 [78] is contact inhibited and independent of exogenous endothelial cell growth factor (ECGF). The cells bind UEA, can take up acLDL and express vWF. Their constitutive expression of ICAM-1, VCAM-1 and E-selectin can be upregulated by TNF-α (BMEC-1) or LPS (TrHBMEC) stimulation. Peripheral blood CD34+ cells adhere to and transmigrate through both BMEC-1 and TrHBMEC just as with primary HBMEC, whereas prostate cancer cells were shown to preferentially adhere to HBME-1 compared to endothelium from other tissues. TrHBMEC furthermore expresses P-selectin, CD31, CD34, CD44, VLA-5 (very late antigen) and ICAM-2. For BMEC-1, the expression of fibronectin receptor, hyaluronate receptor, several integrins, endoglin, collagen VI, CD58 and CD61, whereas for HBME-1 the expression of vimentin was demonstrated. The latter cell type is able to form tubular structures on Matrigel.

Pathologic endothelium

Heffelfinger et al. [79] postulated that the cell line SK HEP-1 is of endothelial origin. SK HEP-1 had been derived from ascites of a patient with liver adenocarcinoma. It was described as being of hepatocellular carcinoma origin. However, Northern blots showed no mRNA for the hepatic-specific proteins albumin, α-fibrinogen or γ-fibrinogen. Instead, electron microscopy showed pinocytotic vesicles, WP-bodies and abundant intermediate filaments identified immunohistochemically as vimentin. The cells did not bind UEA, but expressed vWF and E-selectin and formed tubes on Matrigel, indicating that the cell line is indeed of endothelial origin.

Hoover et al. [80] established a cell line (HAEND) from a liver angiosarcoma of a mine worker who had been exposed to gaseous vinyl chloride 40 years before onset and had redeveloped the angiosarcoma after liver transplantation. HAEND have no cobblestone morphology but are spindle shaped, a morphological feature seen in angiosarcomas, have lost contact inhibition and do not require gelatin, fibronectin or serum to grow. They are positive for vWF, ICAM-1, TF, complement receptor 3 (CR3), HLA class I, but negative for HLA class II. There was low or no expression of E-selectin, VCAM-1, B7, Fc-receptor, CD19, CD20 and CR2.

Summary (immortalized EC)

At present there is a vast choice of immortalized EC lines. The most frequently used and therefore thoroughly characterized cell lines are EA.hy926 [14] and ECV304 [40], of which the latter was recently demonstrated not to be of endothelial origin [42]. Still other new cell lines have been established since the first description of those two lines. IVEC [48] for example, shows a high degree of similarity to primary HUVEC in expression and upregulation of endothelial markers, but contains SV40 genes, whereas C11STH [52] shows similar properties but is derived from a spontaneous mutational event. The micro- and macro-vascular hTERT lines [56] carry many primary EC traits without showing a transformed phenotype. There are also specialized EC lines such as the blood–brain barrier cell line SV-HCEC [64], the liver sinusoid line iSEC [65] or the bone marrow-derived lines BMEC-1 [76] and TrHBMEC [77]. The microvascular cell line HMEC-1 [63] is the first microvascular cell line widely used for different endothelial research purposes and carries many traits of primary microvascular EC.

Lidington et al. [81] compared the three popular EC lines HMEC-1 [63], ECV304 [40] and EA.hy926 [14] with primary HUVEC. After comparing constitutive and induced expression of surface antigens involved in interaction with lymphocytes and monocytes as well as transendothelial migration, the authors stated that HMEC-1 was the most and ECV304 the least similar to primary endothelium. This along with the fact that ECV304 is tumorigenic and not even of endothelial origin [40, 42] seem to make ECV304 a rather unattractive *in vitro* EC model.

Conclusions

Different experiments require different properties of EC. The cell lines reviewed here are of human origin and have been given an extended life span or were immortalized. For some experiments primary cell cultures are a prerequisite. Although primary cultures are less well defined and differ from one isolation to the other, they probably present more primary endothelial traits. In recent years the use primary cells has become more attractive as there is a vast, and still growing, range of commercially available primary ECs from almost any vascular origin. Commercial primary EC circumvent the problems of laborious EC isolation and it is usually possible to purchase EC from the same original batch at

a later moment if further experiments are required. However, the short life span of primary cells cannot be overcome. Further Asahara et al. [73] have presented a technique to isolate EC precursors from peripheral blood that differentiate to EC *in vitro* and *in vivo*.

Another alternative would be the use cell lines or primary cultures of animal origin. The results obtained with animal cells may be more easily compared with *in vivo* data from experiments with the same species. The following references give an example of the broad range of available animal endothelial models. Immortal mouse cell lines were derived from endotheliomas [82] and the myocard [83]. Primary mouse EC were isolated from brain, ovary and lung [84] or pathologic mouse endothelium [85]. An immortalized rat bone marrow EC line [86], primary rat heart EC [87] and immortalized rat brain EC [88] were described. Bovine primary aortic EC [89], fetal bovine bone EC [90] as well as immortalized bovine brain capillary EC [91] have been isolated. Other authors describe immortalized porcine aortic [92] and liver EC [93], as well as primate (rhesus monkey) primary corpus luteum EC [94].

If one chooses human cells, the requirements for a human EC cell line should be:

1. a life span according to the duration of the experiment;
2. stable expression of at least one or two specific endothelial markers characterizing the cells as EC (i.e. presence of WP-bodies, vWF-expression, UEA binding, acLDL uptake, ACE activity, cobblestone morphology);
3. stable expression of the experimentally relevant markers;
4. as few as possible indications for tumorigenic conversion of the cell line (i.e. unstable or aneuploid karyotype, growth in soft agar, tumorigenicity in nude mice).

According to these criteria some cell lines are more useful than others: for specific fields of interest (eg. Kaposi Sarcoma, blood–brain barrier) cell lines derived from the target vessels are the options of choice. For investigating healthy endothelium in an *in vitro* situation closely modelling the *in vivo* status of the majority of blood vessels, physiological endothelial cells derived from vasculature that is not too specialized are superior. The only cell line with an extended life span showing satisfactory expression of endothelial traits without too many negative transformation-related properties is the microvascular cell line HPEC-A1 [30]. Many cell lines with an extended life span were either not well characterized or showed disadvantageous traits such as tumorigenicity or chromosomal instability. They also showed loss of primary endothelial features. Transformed cells with an extended life span would therefore not present major advantages compared to primary cells.

Using an immortalized cell line, the main selection criterium consists of the vessel size of which the EC line was derived. If large-vessel endothelium is to be investigated one would probably choose EA.hy926 [14] which is well characterized. Newer macrovascular cell lines as IVEC [48], C11STH [52] or the hTERT-lines [56] may have the same properties but are not so well characterized yet. As ECV304 have been shown not to be of endothelial origin [42] the choice would probably be EA.hy926. If microvascular endothelium is to be investigated, HMEC-1 [63] and hTERT-HDMEC [56] seem to be the most suitable. Both cell lines bear many primary microvascular features, the former being more well characterized. Investigators looking for 'an endothelial cell line' no matter whether micro- or macrovascular, might consider using a microvascular cell line as by far the most EC in our body are part of the microvasculature.

Yet, it is clear that there are no established superior models for endothelial research *in vitro*. As vasculature is such a complex system, knowledge about interactions within and among EC and their surrounding cells can be gathered by implementing the results of *in vitro* research to animal models (e.g. transgenes, knock-ins and knock-outs) to see effects on the whole vasculature, thereafter going back to *in vitro* work to study the underlying mechanisms in physiology and pathology. As the interest in blood vessels is still growing, we can expect a flow of new EC lines and experimental models presenting increasingly good properties in the future.

References

1. Hohenwarter O, Jakoubek A, Schmatz C, Katinger H. Expression of SV40 tumor antigens enables human endothelial cells to grow independently from foetal calf serum end exogenous growth factors. J Biotechnol 1994; 34: 205–11.
2. Cines DB, Pollak ES, Buck CA et al. Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood 1998; 91(10): 3527–61.
3. Bachetti T, Morbidelli L. Endothelial cells in culture: A model for studying vascular functions. Parmacol Res 2000; 42(1): 9–19.
4. Ruoslahti E, Rajotte D. An address system in the vasculature of normal tissues and tumors. Annu Rev Immunol 2000; 18: 813–27.
5. Pinkney JH, Stehouwer CDE, Coppack SW, Yudkin JS. Endothelial dysfunction: Cause of the insulin resistance syndrome. Diabetes 1997; 46(Suppl 2): S9–13.
6. Gallagher GL, Sumpio BE. Endothelial Cells. Mt. Kisco, New York: Futura Publishing Company 1997; 151–86.
7. Suri C, Jones PF, Patan S et al. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell 1996; 87(7): 1171–80.
8. Jaffe EA, Nachman RL, Becker CG, Minick CR. Culture of human endothelial cells derived from umbilical veins. identification by morphologic and immunologic criteria. J Clin Invest 1973; 52: 2745–56.
9. Watson CA, Camera-Benson L, Palmer-Crocker R, Pober JS. Variability among human umbilical vein endothelial cultues. Science 1995; 268: 447–8.
10. Schütz M, Friedl P. Isolation and cultivation of endothelial cells derived from human placenta. Eur J Cell Biol 1996; 71: 395–401.
11. Folkman J, Haudenschild CC, Zetter BR. Long-term culture of capillary endothelial cells. Proc Natl Acad Sci USA 1979; 76(10): 5217–21.

12. Weibel ER, Palade GE. New cytoplasmic components in arterial endothelia. J Cell Biol 1964; 23: 101–12.
13. Ewenstein BM, Warhol MJ, Handin RI, Pober JS. Composition of the von Willebrand factor storage organelle (Weibel–Palade body) isolated from cultured human umbilical vein endothelial cells. J Cell Biol 1987; 104: 1423–33.
14. Edgell CJS, McDonald CC, Graham JB. Permanent cell line expressing human factor VIII-related antigen established by hybridization. Proc Natl Acad Sci USA 1983; 80: 3734–7.
15. Pober JS, Gimbrone MA Jr, Lapierre LA et al. Overlapping patterns of activation of human endothelial cells by interleukin-1, tumor necrosis factor and immune interferon. J Immunol 1986; 137(6): 1893–986.
16. Dejana E. Endothelial adherens junctions: Implications in the control of vascular permeability and angiogenesis. J Clin Invest 1996; 98(9): 1949–53.
17. Holthöfer H, Virtanen I, Kariniemi AL et al. *Ulex europaeus* I lectin as a marker for vascular endothelium in human tissues. Lab Invest 1982; 47(1): 60–6.
18. Voyta JC, Via DP, Butterfield CE, Zetter BR. Identification and isolation of endothelial cells based on their increased uptake of acetylated-low density lipoprotein. J Cell Biol 1984; 99: 2034–40.
19. Johnson AR, Erdös EG. Metabolism of vasoactive peptides by human endothelial cells in culture: Angiotensin I converting enzyme (kininase II) and angiotensinase. J Clin Invest 1977; 59: 684–95.
20. Michael A Gimbrone J, Fareed GC. Transformation of cultured human vascular endothelium by SV40 DNA. Cell 1976; 9(2): 685–93.
21. Ide H, Minamishima Y, Eizuru Y et al. 'Transformation' of human endothelial cells by SV40 virions. Microbiol Immunol 1988; 32(1): 45–55.
22. Salahuddin SZ, Nakamura S, Biberfeld P et al. Angiogenic properties of Kaposi's sarcoma-derived cells after long-term culture *in vitro*. Science 1988; 242: 430–3.
23. Werner S, Hofschneider PH, Stürzl M et al. Cytochemical and molecular properties of simian virus 40 transformed Kaposi's sarcoma-derived cells: Evidence for the secretion of a member of the fibroblast growth factor family. J Cell Physiol 1989; 141: 490–502.
24. Corbeil J, Evans LA, Vasak E et al. Culture and properties of cells derived from Kaposi sarcoma. J Immunol 1991; 146(9): 2972–76.
25. Iijima S, Ishida M, Nakajima-Iijima S et al. Immortalization of human endothelial cells by origin-defective simian virus 40 DNA. Agric Biol Chem 1991; 55(11): 2847–53.
26. Fickling SA, Tooze JA, Whitley GSJ. Characterization of human umbilical vein cell lines produced by transfection with the early region of SV40. Exp Cell Res 1992; 201: 517–21.
27. Hohenwarter O, Zinser E, Schmatz C et al. Influence of transfected SV40 early region on growth and differentiation of human endothelial cells. J Biotechnol 1992; 25: 349–56.
28. Hohenwarter O, Schmatz C, Katinger H. Stability of von Willebrand factor secretion in different human endothelial hybrid cell lines. Cytotechnology 1992; 8: 31–7.
29. Lassalle P, LaGrou C, Delneste Y et al. Human endothelial cells transfected by SV 40 T antigens: Characterization and potential use as a source of normal endothelial factors. Eur J Immunol 1992; 22: 425–31.
30. Schütz M, Teifel M, Friendl P. Estalishment of a human placental endothelial cell line with extended life span after transfection with SV 40 T-antigens. Eur J Cell Biol 1997; 74(4): 315–20.
31. Hansen TN, Dawson PE, Brockbank KG. Effects of hypothermia upon endothelial cells: Mechanisms and clinical importance. Cryobiology 1994; 31: 101–6.
32. Lieber M, Smith B, Szakal A et al. A continuous tumor cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. Int J Cancer 1976; 17: 62–70.
33. Thornhill MH, Li J, Haskard DO. Leukocyte endothelial cell adhesion: A study comparing human umbilical vein endothelial cells and the endothelial cell line EA-hy-926. Scand J Immunol 1993; 38: 279–86.
34. van Leeuwen EBM, Veenstra R, Wijk RV et al. Characterization of immortalized human umbilical and iliac vein endothelial cell lines after transfection with SV40 large T-antigen. Blood Coagul Fibrinolysis 2000; 11(1): 15–25.
35. Meri S, Mattila P, Renkonen R. Regulation of CD59 expression on the human endothelial cell line EA.hy962. Eur J Immunol 1993; 23: 2511–6.
36. Brown KA, Vora A, Biggerstaff J et al. Application of an immortalized human cell line to the leucocyte–endothelial aherence assay. J Immunol Methods 1993; 163: 13–22.
37. Burke-Gaffney A, Hellewell PG. Regulation of ICAM-1 by dexamethasone in a human vascular endothelial cell line EAhy926. Am J Physiol 1996; 270(2Pt1): C552–61.
38. Kita M, Eguchi K, Kawabe Y et al. Staphylococcal enterotoxin b-specific adhesion of murine splenic T cells to a human endothelial cell line. Immunology 1996; 88: 441–6.
39. Walkden BJ, Turner AJ. Expression of ECE and related membrane peptidases in the EA.hy926 cell line. J Cardiovasc Pharmacol 1995; 26(Suppl 3): S59–60.
40. Takahashi K, Sawasaki Y, Hata JI et al. Spontaneous transformation and immortalization of human endothelial cells. In Vitro Cell Dev Biol 1990; 25: 256–74.
41. Kiessling F, Kartenbeck J, Haller C. Cell–cell contacts in the human cell line ECV304 exhibit both endothelial and epithelial characteristics. Cell Tissue Res 1999; 297(1): 131–40.
42. Brown J, Reading SJ, Jones S et al. Critical evaluation of ECV304 as a human endothelial cell model defined by genetic analysis and functional responses: A comparison with the human bladder cancer derived epithelial cell line t24/83. Lab Invest 2000; 80(1): 37–45.
43. Kobayashi M, Kondo M, Mitsui Y. Establishment of human endothelial cell lines in a serum-free culture and its application for expression of transfected prepro endothelin gene. Hum Cell 1991; 4(4): 296–305.
44. López-Pedrera C, Jardí M, Inglès-Esteve J et al. Characterization of tissue factor expression on the human endothelial cell line ECV304. Am J Hematol 1997; 56(2): 71–8.
45. Takahashi K, Sawasaki Y. Rare spontaneously transformed human endothelial cell line provides useful research tool. In Vitro Cell Dev Biol 1992; 28A: 380–2.
46. Haller C, Kiessling F, Kübler W. Polarized expression of heterologous membrane proteins transfected in a human endothelial-derived cell line. Eur J Cell Biol 1998; 75(4): 353–61.
47. Faller DV, Kourembanas S, Ginsberg D et al. Immortalization of human endothelial cells by murine sarcoma viruses, without morphologic transformation. J Cell Physiol 1988; 134: 47–56.
48. Schwartz B, Vicart P, Delouis C, Paulin D. Mammalian cell lines can be efficiently established in vitro upon expression of the SV40 large T antigen driven by a promotor sequence derived from the human vimentin gene. Biol Cell 1991; 73: 7–14.
49. Vicart P, Testut P, Schwartz B et al. Cell adhesion markers are expressed by a stable human endothelial cell line transformed by the SV40 large T antigen under vimentin promoter control. J Cell Physiol 1993; 157: 41–51.
50. Sasaguri Y, Yanagi H, Nagase H et al. Collagenase production by immortalized human aortic endothelial cells infected with simian virus 40. Virchows Arch B Cell Pathol 1991; 60: 91–7.
51. Kato S, Sasaguri Y, Azagami S et al. Ambient pressure stimulates immortalized human aortic endothelial cells to increase DNA synthesis and matrix metalloproteinase 1 (tissue collagenase) production. Virchows Arch 1994; 425: 385–90.
52. Cockerill GW, Meyer G, Noack L et al. Characterization of a spontaneously transformed human endothelial cell line. Lab Invest 1994; 71(4): 497–509.
53. Fontijn R, Hop C, Brinkman HJ et al. Maintenance of vascular endothelial cell-specific properties after immortalization with an amphotrophic replication-deficient retrovirus containing human papilloma virus 16 e5/e7 DNA. Exp Cell Res 1995; 216: 199–207.

54. Moldovan F, Soliman HR, Bennani H et al. Propriétés fonctionelles d'une nouvelle lignée de cellules endothéliales humaines immortalisées. C R Acad Sci III 1995; 318: 951–8.
55. Tonquèze ML, Jamin C, Böhme M et al. Establishment and characterization of permanent human endothelial cell clones. Lupus 1996; 5: 103–12.
56. Yang J, Chang E, Cherry AM et al. Human endothelial cell life extension by telomerase expression. J Biol Chem 1999; 274(37): 26141–8.
57. Davison PM, Bensch K, Karasek MA. Isolation and growth of endothelial cells from the microvessels of the newborn human foreskin in cell culture. J Invest Dermatol 1980; 75: 316–21.
58. Sherer GK, Fitzharris TP, Faulk WP, LeRoy EC. Cultivation of microvascular endothelial cells from human preputial skin. In Vitro 1980; 16(8): 675–84.
59. Sherer GK, Fitzharris TP, LeRoy EC. Morphologic and functional characterization of cultured microvascular endothelial cells obtained from human preputial skin. In Vitro 1979; 15: 201–2.
60. Swerlick RA, Lee KH, Wick TM, Lawley TJ. Human dermal microvascular endothelial but not human umbilical vein endothelial cells express CD36 *in vivo* and *in vitro*. J Immunol 1992; 148(1): 78–83.
61. Karasek MA. Microvascular endothelial cell culture. J Invest Dermatol 1989; 93: 33S–38S.
62. Kern PA, Knedler A, Eckel RH. Isolation and culture of microvascular endothelium from human adipose tissue. J Clin Invest 1983; 71: 1822–9.
63. Ades EW, Candal FJ, Swerlick RA et al. HMEC-1: Establishment of an immortalized human microvascular endothelial cell line. J Invest Dermatol 1992; 99: 683–90.
64. Muruganandam A, Herx LM, Monette R et al. Development of immortalized human cerebromicrovascular endothelial cell line as an in vitro model of the human blood-brain barrier. FASEB J 1997; 11(13): 1187–97.
65. Hering S, Griffin BE, Strauss M. Immortalization of human fetal sinusoidal liver cells by polyoma virus large T antigen. Exp Cell Res 1991; 195: 1–7.
66. Xu Y, Swerlick RA, Sepp N et al. Characterization of expression and modulation of cell adhesion molecules on an immortalized human dermal microvascular endothelial cell line (HMEC-1). J Invest Dermatol 1994; 102(6): 833–7.
67. Pruckler JM, Lawley TJ, Ades EW. Use of a human microvascular endothelial cell line as a model system to evaluate cholesterol uptake. Pathobiology 1993; 61: 283–7.
68. Candal FJ, Bosse DC, Vogler WR, Ades EW. Inhibition of induced angiogenesis in a human microvascular endothelial cell line by ET-18-OCH3. Cancer Chemother Pharmacol 1994; 34: 175–8.
69. Robinson KA, Candal FJ, Scott NA, Andes EW. Seeding of vascular grafts with an immortalized human dermal microvascular endothelial cell line. Angiology 1995; 46(2): 107–13.
70. Scott NA, Candal FJ, Robinson KA, Ades EW. Seeding of intracoronary stents with immortalized human microvascular endothelial cells. Am Heart J 1995; 129(5): 860–6.
71. Kamal K, Du W, Mills I, Sumpio BE. Antiproliferative effect of elevated glucose in human microvascular endothelial cells. J Cell Biochem 1998; 71(4): 491–501.
72. Budworth RA, Anderson M, Clothier RH, Leach L. Histamine-induced changes in the actin cytoskeleton of the human microvascular endothelial cell line HMEC-1. Toxicol In Vitro 1999; 13(4–5), 789–95.
73. Asahara T, Murohara T, Sullivan A et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997; 275: 964–7.
74. Asahara T, Takahashi T, Masuda H et al. VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. EMBO J 1999; 18(14): 3964–72.
75. Asahara T, Masuda H, Takahashi T et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res 1999; 85: 221–8.
76. Candal FJ, Rafii S, Parker JT et al. BMEC-1: A human bone marrow microvascular endothelial cell line with primary cell characteristics. Microvascr Res 1996; 52: 221–34.
77. Schweitzer KM, Vicart P, Delouis C et al. Characterization of a newly established human bone marrow endothelial cell line: Distinct adhesive properties for hematopoietic progenitors compared with human umbilical vein endothelial cells. Lab Invest 1997; 76(1): 25–36.
78. Lehr JE, Pienta KJ. Preferential adhesion of prostate cancer cells to a human bone marrow endothelial cell line. J Natl Cancer Inst 1998; 90(2): 118–23.
79. Heffelfinger SC, Hawkins HH, Barrish J et al. SK HEP-1: A human cell line of endothelial origin. In Vitro Cell Dev Biol 1992; 28A: 136–42.
80. Hoover ML, Vétvica V, Hoffpauir JM, Tamburro CH. Human endothelial cell line from an angiosarcoma. In Vitro Cell Dev Biol 1993; 29A: 199–202.
81. Lidington EA, Moyes DL, McCormack AM, Rose ML. A comparison of primary endothelial cells and endothelial cell lines for studies of immune interactions. Transplant Immunol 1999; 7: 239–46.
82. Obeso J, Weber J, Auerbach R. A hemangioendotheliaoma-derived cell line: Its use as a model for the study of endothelial cell biology. Lab Invest 1990; 63(2): 259–69.
83. Plendl J, Sinowatz F, Auerbach R. A transformed murine myocardial vascular endothelial cell clone: Characterization of cells *in vitro* and of tumors derived from clone *in situ*. Virchows Arch 1995; 426(6): 619–28.
84. Auerbach R, Alby L, Morrissey LW et al. Expression of organ-specific antigens on capillary endothelial cells. Microvasc Res 1985; 29: 401–11.
85. Moyer CF, Huggins E, Saraptopoulos S et al. Cloned endothelium from autoimmune vascular disease retain structural and functional characteristics of normal endothelial cells. Exp Cell Res 1992; 199: 63–73.
86. Yamazaki K, Lehr JE, Rhim JS, Pienta KJ. Establishment of an immortalized Copenhagen rat bone marrow endothelial cell line. In Vivo 1996; 10(4): 459–62.
87. Diglio CA, Grammas P, Giacomelli F, Wiener J. Rat heart-derived endothelial and smooth muscle cell cultures: Isolation, cloning and characterization. Tissue Cell 1988; 20(4): 477–92.
88. Regina A, Romero IA, Greenwood J et al. Dexamethasone regulation of p-glycoprotein activity in an immortalized rat brain endothelial cell line GPNT. J Neurochem 1999; 73(5): 1954–63.
89. Lokeshwar VB, Iida N, Bourguignon LYW. The cell adhesion molecule GP116, is a new CD44 variant (ex14/v10) involved in hyaluronic acid binding and endothelial cell proliferation. J Biol Chem 1996; 271(39): 23853–64.
90. Streeten EA, Ornberg R, Curcio F et al. Cloned endothelial cells from fetal bovine bone. Proc Natl Acad Sci USA 1989; 86(3): 916–20.
91. Durieu-Trautman O, Foignant-Chaverot N, Pedomo J et al. Immortalization of brain capillary endothelial cells with maintenace of structural characteristsics of the blood–brain barrier endothelium. In Vitro Cell Dev Biol 1991; 27A: 771–8.
92. Maher SE, Karmann K, Min W, Hughes CC, Pober JS, Brothwell AL. Porcine endothelial CD86 is a major costimulator of xenogenic human T cells: Cloning, sequencing, and functional expression in human endothelial cells. J Immunol 1996; 157(9): 3838–44.
93. Sepp A, Skacel P, Lindstedt R, Lechler RI. Expression of alpha-1,3-galactose and other type 2 oligosaccharide structures in a porcine endothelial cell line transfected with human alpha-1,2-fucosyltransferase cDNA. J Biol Chem 1997; 272(37): 23104–10.
94. Christenson LK, Stouffer RL. Isolation and culture of microvascular endothelial cells from the primate corpus luteum. Biol Reprod 1996; 55: 1397–1404.