Modeling human endothelial cell transformation in vascular neoplasias

Victoria W. Wen¹ and Karen L. MacKenzie¹,*

Endothelial cell (EC)-derived neoplasias range from benign hemangioma to aggressive metastatic angiosarcoma, which responds poorly to current treatments and has a very high mortality rate. The development of treatments that are more effective for these disorders will be expedited by insight into the processes that promote abnormal proliferation and malignant transformation of human ECs. The study of primary endothelial malignancy has been limited by the rarity of the disease; however, there is potential for carefully characterized EC lines and animal models to play a central role in the discovery, development and testing of molecular targeted therapies for vascular neoplasias. This review describes molecular alterations that have been identified in EC-derived neoplasias, as well as the processes that underpin the immortalization and tumorigenic conversion of ECs. Human EC lines, established through the introduction of defined genetic elements or by culture of primary tumor tissue, are catalogued and discussed in relation to their relevance as models of vascular neoplasia.

Introduction
Vascular neoplasias are a spectrum of disorders that include rare, aggressive malignancies such as angiosarcoma, as well as commonly occurring infantile hemangiomas that are usually indolent but can give rise to life-threatening complications when vital organs are involved (Drolet et al., 1999; Szajerka and Jablecki, 2007; Koch et al., 2008; Young et al., 2010). The unifying pathological feature of these conditions is perturbation of endothelial cell (EC) proliferation and disorganization of endothelial tissue architecture. In a non-pathological state, mature ECs have limited proliferative ability and form an organized monolayer along the inner surface of blood and lymphatic vessels. These cells are derived from a hierarchy of highly proliferative progenitors that are thought to reside within the bone marrow and are ontologically related to hematopoietic stem cells (Asahara et al., 1997; Asahara et al., 1999). An array of cytokines and chemokines direct the recruitment and homing of endothelial progenitors from the bone marrow, via the peripheral circulation, to the inner walls of blood and lymph vessels where they differentiate and contribute to many normal physiological processes, such as wound healing, inflammation, coagulation, cell migration and hematopoiesis (Shi et al., 1998; Asahara et al., 1999; Krishnaswamy et al., 1999; Takahashi et al., 1999).

The mitogenic signaling pathways that regulate the proliferation of normal ECs have been intensively studied and are reviewed elsewhere (Carmeliet and Jain, 2011). However, relatively few investigations have focused on the molecular mechanisms that promote unregulated proliferation and transformation of ECs during the development of vascular neoplasia. This is in part due to the rarity of EC-derived malignancies as well as the focus of most investigations on end-stage disease, which precludes study of the molecular events involved in the initiation and progression of disease. Consequently, little progress has been made in the development of novel therapeutics for the treatment of endothelial malignancies over the past few decades. By describing the molecular features of vascular neoplasias, as well as the cell lines and in vivo models that are available to model EC transformation, the aim of this review is to inform and motivate preclinical studies of new therapeutic approaches to the treatment of specific EC neoplasias.

Clinical and molecular features of EC-derived neoplasias
Hemangioma
Hemangioma is the most common tumor of infancy, affecting ~10% of Caucasian neonates (Table 1) (Drolet et al., 1999). This neoplasia develops as a rapidly growing disorganized mass of ECs. Most hemangiomas appear within the first few weeks of birth and are characterized by an initial period of rapid tumor growth that is driven by the proliferation of immature ECs. This is followed by a ‘rest phase’, in which there is little change in the appearance of the tumor. A subsequent slow involuting phase associated with EC differentiation and apoptosis marks regression of the tumor in almost all cases. The involuted tumor is replaced by fibrous and fatty tissue. Although histologically benign, non-invasive and unlikely to progress to a malignancy, visceral hemangiomas can result in substantial morbidity and mortality by causing obstruction, hemorrhage or by diverting blood flow. Currently available treatments for life-threatening or otherwise complicated cases of hemangioma have limited efficacy and commonly result in treatment-associated toxicity.

¹Cancer Cell Development Group, Children's Cancer Institute Australia for Medical Research, Lowy Cancer Research Centre, University of New South Wales, Randwick, NSW, Australia
*Author for correspondence (k.mackenzie@unsw.edu.au)

© 2013. Published by The Company of Biologists Ltd
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.
Table 1. Characteristics of vascular neoplasias

| EC tumors                      | Type of malignancy | Prevalence                           | Typical age of onset (years) | Molecular pathways involved in disease pathogenesis | Karyotype * | References                          |
|--------------------------------|---------------------|--------------------------------------|-------------------------------|-----------------------------------------------------|-------------|-------------------------------------|
| Hemangioma                     | Benign              | Common (~10% of Caucasian newborns)  | <1.5                          | Normal (p16INK4a, p53, VEGF, cyclin D1, NFκB)       | Simple      | Drolet et al., 1999; Truss et al., 2006; Jinnin et al., 2008; Mohamed et al., 2009; Pareja et al., 2009; Greenberger et al., 2010 |
| Hemangioendothelioma            | Benign and malignant (intermediate) | Rare (<0.0001%) in general population but common (~20-60%) in AIDS-infected individuals | >5                            | Not evaluated (p16INK4a, p53, VEGF, mTOR, telomerase) | Simple or unstable | Theurillat et al., 2003; He et al., 2006; Marucci et al., 2006; Tsarouha et al., 2006 |
| Kaposi's sarcoma (arise from KSHV infection) | Benign and malignant (intermediate) | Rare (~1% of all soft tissue sarcomas) | >25                           | Abnormal (inactivated by LANA)                      | Simple or complex | Gnep et al., 1984; Friberg et al., 1999; Verna et al., 2004; Si and Robertson, 2006; Sodhi et al., 2006; Szajerka and Jablecki, 2007 |
| Angiosarcoma (or hemangiosarcoma) | Malignant           | Rare (~1% of all soft tissue sarcomas) | >70                           | Abnormal (p14ARF, Mdm-2, K-ras, H-ras)              | Simple, complex or unstable | Mark et al., 1996; Przygodzki et al., 1997; Ziet et al., 1998; Boivin-Angèle et al., 2000; Garcia et al., 2000; Tannapfel et al., 2001; Wong et al., 2001; Zu et al., 2002; Wehrhauch et al., 2002; Baumhoer et al., 2005; Itakura et al., 2008 |

* A simple karyotype is characterized by a near-diploid chromosome number and two or fewer karyotypic abnormalities. A complex karyotype is defined as five or more chromosomal aberrations. An unstable karyotype is characterized by a complex karyotype with a high frequency of random aberrations and numerous non-clonal marker and ring chromosomes.

EC, endothelial cell; KSHV, Kaposi's sarcoma-associated herpes virus (also known as HHV-8 [human herpes virus type 8]); VEGF, vascular endothelial growth factor; AIDS, acquired immunodeficiency syndrome; LANA, latency-associated nuclear antigen.

Intermediate grade vascular malignancies

Hemangioendotheliomas and Kaposi’s sarcoma (KS) are rare tumors that have been identified as intermediate-grade endothelial tumors that are ranked between benign hemangioma and aggressive angiosarcoma.KS encompasses a group of neoplasms in which the lesions are composed of proliferating spindle cells that stain positively for factors associated with the vascular endothelium and exhibit nuclear atypia. The high incidence of KS in AIDS patients suggests that KS is caused by HHV-8, which is a human herpes virus type 8.

Hemangiomas are composed of morphologically normal, immature ECs that are clonally derived and exhibit enhanced proliferation and migration (Mulliken et al., 1982; Boye et al., 2001). Hemangiomas are driven by an imbalance in angiogenic signaling factors and activation of nuclear factor-κB (NFκB) signaling. Insights into the molecular events resulting from the cyto genetic alterations have been identified in this disorder. Chromosomal abnormalities that have been observed in hemangiomas include loss of heterozygosity at chromosome regions 5q, 14q and 17p13, as well as loss of the Y chromosome and amplification of the cyclin D1 gene on chromosome 11. FIH tumor suppressor pathway have been shown to be valuable for the development of improved therapies for these disorders.
relatively indolent disease that is rarely life-threatening and is often left untreated. Indeed, spontaneous remissions have been reported (Brooks, 1986). Cases of KS that do progress usually involve local invasion, whereas distant dissemination is extremely rare. KS also manifests in association with immune suppression in individuals with AIDS and organ-transplant recipients (Gnepp et al., 1984). In contrast to classic KS, AIDS-associated KS presents as a more widespread, multifocal disorder where life-threatening complications can result from visceral involvement.

KS arises in individuals infected with human herpes virus 8 [also known as KS-associated herpes virus (KSHV)] (Chang et al., 1994). Since the discovery of KSHV, there has been considerable investigation of the genetics and oncogenic potential of this virus (reviewed by Ganem, 2010). The KSHV genome encodes viral homologs of cellular genes that promote the cell cycle and cellular immortalization, inhibit apoptosis, stimulate angiogenesis, and enable infected cells to evade the immune system (Boshoff et al., 1995; Bais et al., 1998; Knight et al., 2001; Bais et al., 2003; Sodhi et al., 2006; Chang et al., 2009). Consequently, activation of cellular oncogenes and mutation of tumor suppressor genes might play a less substantial role in the initiation of KS relative to other sarcomas. Although TP53 (encoding p53) mutations seem to be rare in KS, p53 protein function can be suppressed through direct interaction with the KSHV-encoded latency-associated nuclear antigen.
Angiosarcoma

Angiosarcoma is a rare malignancy that makes up less than 1% of all sarcomas. In contrast to other EC-derived neoplasias, angiosarcomas are highly malignant (Table 1; Fig. 1) (Young et al., 2010). This malignancy occurs with a peak incidence in the seventh decade of life and tends to arise in the viscer, skin and soft tissue. Angiosarcomas have a propensity to recur locally and spread widely, exhibiting a high rate of lymph node and systemic metastases. The disease is associated with a high mortality rate as a result of poor response to treatment (Mark et al., 1996; Lezama-del Valle et al., 1998). With a reported 5-year survival rate of ~20% and very little change in treatment options over the past decades, innovative therapies are urgently needed. Tumor resection with adjuvant radiotherapy is currently the main treatment approach to angiosarcoma, although a few groups have recently begun to pursue biological and molecular targeted approaches (Koontz et al., 2008; George et al., 2009; Maki et al., 2009; Young et al., 2010).

A more detailed understanding of the molecular pathways involved in malignant transformation of ECs and more extensive preclinical studies will be invaluable for the validation of these therapies and the optimization of effective therapeutic strategies.

Most cases of angiosarcoma arise sporadically; however, previous irradiation, exposure to toxic chemicals and chronic lymphedema are known risk factors (Mark et al., 1996; Lezama-del Valle et al., 1998). Activation of the oncogene K-ras through acquisition of specific point mutations is a common event in angiosarcoma, reportedly occurring in 29% of hepatic angiosarcomas (7 out of 24) and 60% of cardiac angiosarcomas (3 out of 5) (Przygodzki et al., 1997; Boivin-ANGèlè et al., 2000; García et al., 2000). The incidence of K-ras mutations seems to be highest among angiosarcomas associated with exposure to vinyl chloride, where mutations have been detected in ~80% of cases (5 out of 6 cases tested) (Boivin-ANGèlè et al., 2000). Ras mutations are likely to contribute to the highly metastatic nature of angiosarcomas, given that they are generally not found in non-malignant vascular neoplasias (Table 1). The malignant growth of angiosarcoma is driven by the proliferation of pleomorphic cells that exhibit varying degrees of EC differentiation and give rise to a chaotic tissue architecture (Young et al., 2010). VEGF is expressed at high levels in angiosarcoma and the expression of VEGF receptors is associated with a more differentiated tumor phenotype and favorable prognosis (Zietz et al., 1998; Itakura et al., 2008). Consistent with those observations, inhibition of VEGF receptor 1 (VEGFR1) has been shown to increase the proliferation of canine angiosarcoma cells in vitro (Tamburini et al., 2009).

Abnormalities in the TP53 pathway – including p53 mutations, Mdm-2 overexpression and inactivation of p14ARF – are common molecular aberrations in angiosarcoma (Hollstein et al., 1994; Zietz et al., 1998; Weihrauch et al., 2002). For example, mutations in the TP53 gene were detected in ~30% of liver angiosarcomas (Przygodzki et al., 1997; Weihrauch et al., 2002). Consistent with the high frequency of p53 dysfunction, complex karyotypes and chromosomal instability are often observed in angiosarcomas. Chromosomal aberrations reported in angiosarcomas include deletions within the CDKN2A locus at chromosome region 9p21, which encodes tumor suppressors p16INK4a, p15INK4b and p14ARF. Inactivation of p16INK4a by promoter methylation is another frequent event in liver angiosarcoma (Tannapfel et al., 2001; Weihrauch et al., 2002). Studies of liver angiosarcoma showed that p16INK4a was repressed by promoter methylation in 63% (12 out of 19) of tumor samples, whereas 5% (1 out of 19) exhibited homozygous deletion and 10% (2 out of 19) exhibited loss of heterozygosity at the CDKN2A locus. Overall, inactivation of p16INK4a was detected in 74% (14 out of 19) of angiosarcomas. The importance of p16INK4a repression in angiosarcoma was also evident from canine studies that showed sustained expression of p16INK4a in 100% of benign hemangiomas (a total of ten were examined), and p16INK4a suppression in 82% (32 out of 39) angiosarcomas (Yonemaru et al., 2007). Together, these studies implicate Ras activation plus aberrations in the p53 pathway and silencing of p16INK4a as key events in the pathogenesis of advanced, but not benign, vascular tumors (Table 1, Fig. 1).

Immortalization and tumorigenic conversion of human ECs

The limited replicative potential of normal human ECs

A fundamental step in the development of endothelial neoplasias is the deregulation of cell proliferation. Normal human cells have a finite replicative ability, yet cancer cells are capable of unlimited replication, i.e. they are immortal. Cellular immortality is considered to be a requirement for malignant transformation and is a defining property of the subset of tumor cells that drive tumor growth and recurrence (Zhou et al., 2009). Previous studies have revealed that the in vitro replicative potential of normal human cells, including ECs, is restricted by both intrinsically and extrinsically initiated stresses that induce senescence (e.g. telomere shortening and an oxidative environment, respectively) (Hayflick and Moorhead, 1961; Johnson and Longenecker, 1982; Yuan et al., 2008). Senescent ECs typically adopt a flattened morphology and demonstrate cytoplasmic spreading, increased granularity, vacuolization and multi-nucleation. They are resistant to mitogenic stimulation and exhibit pH-dependent β-galactosidase activity (Johnson and Longenecker, 1982; Dimri et al., 1995).

It is well established that the shortening of chromosomal-end structures, referred to as telomeres, plays a central role in the onset of senescence (Artandi and DePinho, 2010). Telomeres normally function to prevent chromosomal end-to-end fusions and to maintain genomic integrity during cell division. However, telomeric DNA is eroded during consecutive cell divisions as a consequence of the inability of DNA polymerases to synthesize the 5’ terminus of linear DNA. Exposure to oxidizing agents accelerates telomere shortening and the onset of EC senescence; conversely, free radical scavengers reduce the rate of telomere shortening (von Zglinicki et al., 1995; Xu et al., 2000; Kurz et al., 2004; Napier et al., 2010). The progressive shortening of telomeres has been demonstrated in serially passaged cultures of human ECs derived from the
umbilical vein, iliac arteries and veins, abdominal aorta, and bone marrow (Chang and Harley, 1995; Aviv et al., 2001; MacKenzie et al., 2002). Telomere shortening has also been demonstrated in arterial ECs in vivo in association with increasing age (Okuda et al., 2000).

When telomeres reach a critically short length, they are prone to fusion and promote chromosome rearrangements. Hence, primary ECs that are maintained in culture for long periods of time frequently harbor aberrant chromosomes (Wagner et al., 2001). In addition to structural rearrangements, changes in whole chromosome number (aneuploidy) tend to occur as cultured ECs age and approach senescence in vitro (Nichols et al., 1987; Johnson et al., 1992; Zhang et al., 2000; Aviv et al., 2001). In normal human cells, chromosome fusions and rearrangements initiate a DNA damage response that culminates in activation of tumor suppressor p53 and transcriptional upregulation of p21Cip1/Waf1, which halts cell cycle progression and initiates senescence (Stein et al., 1999). Tumor suppressor p16INK4a is subsequently upregulated, resulting in hypo-phosphorylation of the retinoblastoma tumor suppressor protein (pRb) and a sustained growth arrest ( Munro et al., 1999; Wagner et al., 2001; Freedman, 2005). The activation of tumor suppressor pathways and induction of senescence plays a crucial role in preventing replication of aged endothelial cells harboring cytogenetic aberrations. Disruption of these mechanisms enables cells to continue to proliferate and initiate neoplastic growth, as outlined below.

**Lifespan extension, immortality and genetic evolution of ECs**

During neoplastic transformation, the replicative lifespan of human cells is extended by either the activation of a telomere maintenance mechanism (described below) or inactivation of a tumor suppressor pathway (reviewed by Reddel, 2000). Inactivation of tumor suppressor pathways enables cells to progress through the cell cycle with critically short telomeres. However, short, dysfunctional telomeres that are present in proliferating cells promote chromosomal fusions and genomic instability (Ray et al., 1990; Counter et al., 1992). Telomere dysfunction in pre-malignant and cancerous cells is typically evidenced by dicentric chromosomes, unbalanced translocations, gene amplifications, deletions and ring chromosomes ( Ducray et al., 1999; Gisselsson et al., 2001; O’Hagan et al., 2002). These types of chromosomal aberrations are frequently observed in KS and angiosarcoma, and have also been identified in immortalized EC lines, particularly those with defective tumor suppressor pathways (Schuborg et al., 1998; Wong et al., 2001; Wen et al., 2006). Chromosomal instability accelerates the rate and accumulation of molecular changes that facilitate malignant transformation and tumor progression (Rudolph et al., 2001), including activation of a telomere maintenance mechanism, oncogene activation or silencing of tumor suppressor genes.

Under standard culture conditions, ECs that bypass senescence usually succumb to a proliferative ‘crisis’ that is characterized by an increased rate of cell death and reduced cell expansion (MacKenzie et al., 2002; Gu et al., 2003; Nisato et al., 2004; Wen et al., 2006). Crisis is thought to be initiated by telomere dysfunction, genetic catastrophe and/or fatal oxidative damage. Cells that are driven into crisis by the deactivation of tumor suppressor pathways resume proliferation if they undergo a spontaneous molecular event that activates a telomere maintenance mechanism: either activation of the enzyme telomerase or an alternate telomere lengthening mechanism (ALT) (Counter et al., 1992; Bryan et al., 1995). Telomere maintenance stabilizes chromosomes and thereby enables both escape from crisis and unlimited proliferation.

**Extension of replicative lifespan and immortalization of ECs following transfection with viral antigens**

The extended replicative lifespan that results from the inactivation of tumor suppressor pathways is thought to represent a premalignant stage of the multistep process of cancer development. Several investigations have demonstrated that the replicative lifespan of normal human ECs derived from bone marrow microvasculature, the umbilical vein and the iliac vein is extended by transfection with Simian virus 40 (SV40) T antigen or human papilloma virus (HPV)-encoded E6 and E7 oncoproteins (Table 2). The large T antigen of SV40 and the HPV E6 and E7 oncogenes functionally inactivate the p53 and pRb tumor suppressor pathways and thereby enable transfected cells to bypass senescence (Girardi et al., 1965; Werness et al., 1990; Shay et al., 1991). Viral-antigen-transfected ECs have been shown to replicate for up to 42 passages beyond senescence (Table 2) (Gimbrone and Fareed, 1976; Schwartz et al., 1991; Fickling et al., 1992; Hohenwarter et al., 1992b; Lassalle et al., 1992; Bailey et al., 1994; Rhim et al., 1998; Krump-Konvalinkova et al., 2001; O’Hare et al., 2001; MacKenzie et al., 2002).

A number of studies also reported that viral transformation leads to immortalization of ECs (Ades et al., 1992, 1999; Fontijn et al., 1995; Burger et al., 1998; Rhim et al., 1998; Rood et al., 2000). However, a growth crisis prior to immortalization was noted in several studies (Ades et al., 1992; Fontijn et al., 1995; Rood et al., 2000; MacKenzie et al., 2002) and two studies showed that telomerase was spontaneously activated in the immortalized ECs (Rhim et al., 1998; Gagnon et al., 2002). Ectopic activation of telomerase was shown to cooperate with SV40 transformation to overcome crisis and efficiently promote immortalization of bone-marrow- and mammary-derived ECs ( O’Hare et al., 2001; MacKenzie et al., 2002).

Together, these studies suggest that inactivation of tumor suppressor pathways and activation of a telomere maintenance mechanism is necessary and sufficient for in vitro immortalization of human ECs.

Immortalization of ECs by transfection with viral oncogenes and activation of telomerase is not sufficient for malignant conversion of ECs (Burger et al., 1998; Rhim et al., 1998; MacKenzie et al., 2002). Nevertheless, viral-antigen-transfected ECs do exhibit some characteristics of transformed cells, including the ability to proliferate independently of exogenous VEGF, and reduced expression of proteins that define the endothelial phenotype, including VEGFR2, platelet endothelial cell adhesion molecule-1 (CD31; also known as PECAM-1), VE cadherin and von Willebrand factor (vWF) (Table 2) (Gimbrone and Fareed, 1976; Hohenwarter et al., 1992b; Rood et al., 2000; MacKenzie et al., 2002; Shao and Guo, 2004). These features parallel the abnormal expression of VEGF receptors and tendency for downregulation of vWF and CD31 in late-stage hemangiomas (Takahashi et al., 1994). Viral-oncogene-transformed ECs also accumulate genomic rearrangements that are similar to those observed in EC malignancies (Gimbrone and Fareed, 1976; MacKenzie et al., 2002).
Telomerase-mediated immortalization of human ECs

The active core of the telomerase holoenzyme is a ribonuclear protein complex that includes a reverse transcriptase (hTERT), a non-coding RNA that functions as a template for telomere synthesis (hTR) and an RNA-binding and -modifying protein, dyskerin, which stabilizes the RNA component (Feng et al., 1995; Nakamura et al., 1997; Cohen et al., 2007). Although telomerase is not expressed in mature somatic cells, its activation accounts for telomere maintenance in ~85% of all human cancers and immortal cell lines. Telomerase activity is also readily detected in human germ cells, stem cells and normal endothelial progenitors (Kim et al., 1994; Wright et al., 1996). Telomerase is downregulated during development, differentiation and with in vitro passage (Hsiao et al., 1997; Ingram et al., 2005). Consequently, it is not usually detectable in differentiated ECs.

Ectopic expression of hTERT reactivates telomerase in mature ECs and is further bolstered by co-expression of hTR (Yang et al., 1999; MacKenzie et al., 2002; Gu et al., 2003; Nisato et al., 2004).

Table 2. Viral-antigen-transformed EC lines

| Tissue source  | Cell line name | Exogenous genes | Other viral gene | Lifespan | CD31 | VEGFR1 | vWF | ICAM-1 | Karyotypeb | References |
|----------------|----------------|-----------------|------------------|----------|------|--------|-----|--------|-----------|------------|
| Bone marrow   | BMSVT, BMEC-1  | + – – – –       | Extended         | + +/–    | +    | Unstable |     |         |            | Candal et al., 1996; MacKenzie et al., 2002 |
| Mammary microvascular | HMME | + – – – – | Extended | NE NE NE NE NE | O'Hare et al., 2001 |
| Dermal microvascular | HMEC-1 | + – – – – | Immortalized | + +/– | + | NE | Ades et al., 1992; Ribeiro et al., 1995; Unger et al., 2002a; Shao and Guo, 2004 |
| Umbilical vein | CS2, Q2, IB10 | + – – – – | Extended | NE NE +/– NE Abnormal | Hohenwarter et al., 1992b; Hohenwarter et al., 1994; Hohenwarter et al., 1997 |
| Umbilical vein | SVHEC-D | + – – – – | Extended | NE NE – – Unstable | Gimbrone and Fareed, 1976 |
| Umbilical vein | EC-pSV1 | + – – – – | Extended | NE NE +/– + NE | Lassalle et al., 1992 |
| Umbilical vein | SGHEC-7 | + – – – – | Extended | NE NE +/– + Abnormal | Fickling et al., 1992 |
| Umbilical vein | Clone 1, Clone 6 | + – – – – | Extended | NE NE + + NE | Bailey et al., 1994 |
| Umbilical vein | EVLC2 | + – – – – | NE | – + – + NE | van Leeuwen et al., 2000; Unger et al., 2002a |
| Umbilical vein | Clone 1, Clone 2 | + – – – – | Extended | NE NE +/– NE NE | Schwartz et al., 1991 |
| Umbilical vein | E7 HUVEC | – – + – | Extended | NE NE NE NE NE | Burger et al., 1998; Rhim et al., 1998 |
| Umbilical vein | E6 HUVEC | – + – – | Extended | NE NE NE NE NE | Burger et al., 1998; Rhim et al., 1998 |
| Pulmonary      | HPMEC | + – – – | Extended | + NE + + NE | Krump-Konvalinkova et al., 2001 |
| Bone marrow    | HBMCEC-27, HBMCEC-28, HBMCEC-33, HBMCEC-60 | – + + – | Immortalized | NE NE + + NE | Rood et al., 2000 |
| Umbilical vein | EC-RF7, 24 | – + + – | 2/24 clones immortalizedc | + NE + + NE | Fontijn et al., 1995 |
| Umbilical vein | PymT-EC | – – – + | Extended | + NE + NE NE | Primo et al., 2000 |

*Characteristics of the EC phenotype, i.e. the direction of the expression change for each of the molecular markers, is explained in the text. A positive sign indicates correspondence with the EC phenotype; a negative sign indicates deviation from the EC phenotype. An unstable karyotype was defined by a rapidly evolving karyotype with a high frequency of random aberrations and numerous non-clonal marker and ring chromosomes. An abnormal karyotype denotes unspecified chromosomal abnormalities or aneuploidy.

*Immortalized via growth crisis.

NE, not evaluated.
Napier et al., 2010). Reactivation of telomerase activity has been shown to extend the replicative lifespan of normal human ECs, but is not sufficient for immortalization. A number of studies have shown that ECs that overexpress hTERT are subject to a growth crisis following an extended period of replication (Krump-Konvalinkova et al., 2001; O’Hare et al., 2001; MacKenzie et al., 2002; Gu et al., 2003; Nisato et al., 2004; Wen et al., 2006; Takano et al., 2008) (Table 3). The occurrence of crisis in hTERT-transduced EC cultures highlights a requirement for additional molecular events to cooperate with telomerase in the immortalization process. The efficient immortalization of ECs co-transfected with hTERT and viral oncogenes is consistent with the inactivation of tumor suppressor genes being a crucial requirement (Table 3) (O’Hare et al., 2001; MacKenzie et al., 2002). This is further supported by studies that showed that hTERT-transduced ECs frequently silenced tumor suppressor p16INK4a, which functions as a major regulator of the RB pathway during hTERT-driven immortalization (Wen et al., 2006). Furthermore, abnormalities in the TP53 pathway have also been reported in hTERT-immortalized EC lines (Wen et al., 2006). This inactivation of tumor suppressor pathways is likely to be required for telomerase-transduced ECs to overcome cellular stress (e.g. oxidative stress) during immortalization.

EC lines immortalized following hTERT transduction retain many of the properties of normal human ECs, including continued expression of normal EC surface markers, including CD31 and ICAM-1, and a requirement for VEGF signaling for continued proliferation (Table 3) (Yang et al., 1999; Krump-Konvalinkova et al., 2001; MacKenzie et al., 2002; Nisato et al., 2004; Shao and Guo,

Table 3. hTERT-mediated immortalization of EC lines

| Tissue source      | Cell line name | Replicative lifespan | EC phenotype | Replicative lifespan | EC phenotype | Karyotype | TSG pathway | References |
|-------------------|----------------|----------------------|--------------|----------------------|--------------|-----------|-------------|------------|
| Aortic            | HAEC-TERT      | Immortalized         | CD31, VEGFR1, vWF, ICAM-1 | +         | NE | + | Simple | NE | + | + | Yang et al., 1999 |
| Aortic            | HAEC           | Immortalized         | NE | NE | NE | NE | NE | NE | NE | Matsushita et al., 2001 |
| Bone marrow       | BMhTERT-1      | Immortalized         | + | + | NE | + | NE | NE | NE | MacKenzie et al., 2002; Wen et al., 2006 |
| Bone marrow       | BMhTERT-2      | Immortalized         | + | + | NE | + | NE | NE | NE | MacKenzie et al., 2002; Wen et al., 2006 |
| Bone marrow       | BMhTERT clones (p16-positive) | Immortalized | + | + | NE | + | NE | NE | NE | Wen et al., 2006 |
| Bone marrow       | BMhTERT clones (p16-negative) | Immortalized | + | + | NE | + | NE | NE | NE | Wen et al., 2006 |
| Bone marrow       | BM2A4          | Immortalized         | + | + | NE | + | NE | NE | NE | Wen et al., 2006 |
| Dermal lymphatic   | TERT-HDLEC     | Extended             | CD31, VEGFR1, vWF, ICAM-1 | +         | NE | NE | NE | NE | NE | Nisato et al., 2004 |
| Dermal microvascular | TIME         | Immortalized         | CD31, VEGFR1, vWF, ICAM-1 | +         | NE | NE | NE | Normal | NE | Venetsanakos et al., 2002 |
| Mammary microvascular | HMME     | Extended             | CD31, VEGFR1, vWF, ICAM-1 | +         | NE | NE | NE | NE | NE | O’Hare et al., 2001 |
| Microvascular     | HMVEC          | Immortalized         | CD31, VEGFR1, vWF, ICAM-1 | +         | NE | NE | NE | NE | NE | Shao and Guo, 2004 |
| Brain microvascular | TERT-HBEC   | Extended             | CD31, VEGFR1, vWF, ICAM-1 | +         | NE | NE | NE | NE | NE | Gu et al., 2003 |
| Dermal microvascular | HDMC-TERT   | Immortalized         | CD31, VEGFR1, vWF, ICAM-1 | +         | NE | NE | NE | Normal | NE | Yang et al., 1999 |
| Umbilical vein    | i-HUVEC        | Immortalized         | CD31, VEGFR1, vWF, ICAM-1 | +         | NE | NE | NE | Abnormal | + | NE | Freedman and Folkman, 2004 |
| Umbilical vein    | HUVEC          | Extended             | CD31, VEGFR1, vWF, ICAM-1 | +         | NE | NE | NE | NE | NE | Takano et al., 2008 |
| Umbilical vein    | HUVEC          | Extended             | CD31, VEGFR1, vWF, ICAM-1 | +         | NE | NE | NE | NE | NE | Young et al., 2003 |
| Pulmonary         | HPMEC          | Extended             | CD31, VEGFR1, vWF, ICAM-1 | +         | NE | NE | NE | NE | NE | Krump-Konvalinkova et al., 2001 |
| Umbilical vein    | HUVEC-TERT     | Immortalized         | CD31, VEGFR1, vWF, ICAM-1 | +         | NE | NE | NE | Simple | + | + | Yang et al., 1999 |
| Coronary arteries | HCAEC-TERT     | Immortalized         | CD31, VEGFR1, vWF, ICAM-1 | +         | NE | NE | NE | Simple | + | + | Yang et al., 1999 |
| Saphenous vein    | HSVGEC-TERT    | Immortalized         | CD31, VEGFR1, vWF, ICAM-1 | +         | NE | NE | NE | Simple | + | + | Yang et al., 1999 |

Characteristics of the EC phenotype, i.e. the direction of the expression change for each of the molecular markers, is explained in the text. A positive sign indicates correspondence with the EC phenotype; a negative sign indicates deviation from the EC phenotype. A simple karyotype is characterized by a near-diploid chromosome number and two or fewer karyotypic abnormalities. A complex karyotype included five or more chromosomal aberrations. An unstable karyotype was defined by a rapidly evolving karyotype with a high frequency of random aberrations and numerous non-clonal marker and ring chromosomes. An abnormal karyotype denotes unspecified chromosomal abnormalities or aneuploidy. Analysis of TP53 and RB tumor suppressor pathways in the late passage EC cells; + denotes presence and – denotes dysfunction of p53-p21 or p16INK4a-pRB pathways; Normal phosphorylation of pRB while p16INK4a status was not evaluated. Immortalized via growth crisis.

EC, endothelial cell; NE, not evaluated; TSG, tumor suppressor gene.
acquisition of metastatic ability (Krump-Konvalinkova et al., 2003). These studies suggest that ALT - clone-derived angiosarcoma cell lines, ISO-HAS.1 and AS-M.5 (evaluated in ISO-HAS and ISO-HAS.1) are some of the most heterogeneous and abnormalities detected in the p16INK4a-negative BMECs were typical of those that have been documented in EC-derived tumors. Specific aberrations that recur in p16INK4a-repressed cell lines and are also observed in angiosarcoma include monosomy 13, loss of the Y chromosome, rearrangements involving 17p, and trisomies 20 and 11 (Mandahl et al., 1990; Kindblom et al., 1991; Gil-Butso et al., 1994; Cerilli et al., 1998; Schuborg et al., 1998; Wong et al., 2001; Zui et al., 2001; Baumeier et al., 2005; Tsarouha et al., 2006; Wen et al., 2006). Repression of p16INK4a and the concurrent development of karyotypic complexity is a feasible depiction of the molecular alterations that permit the accumulation of genomic imbalances in EC neoplasias.

Relatively little is known about the mechanism(s) that support telomere maintenance in the various types of human vascular neoplasias in patients. However, one study demonstrated telomerase activity in 22 out of 22 KS lesions (Chen et al., 2001), and others have shown that HSV-encoded LANA directly activates Sp1-mediated transcription of hTERT (Knight et al., 2001; Verma et al., 2004). In contrast, an assessment of telomerase activity in canine angiosarcomas showed no detectable telomerase enzyme activity in 6 out of 7 cases (Yazawa et al., 1999; Chandler et al., 2009). Telomerase activity was also undetectable in two single-clone-derived angiosarcoma cell lines, ISO-HAS.1 and AS-M.5 (Krump-Konvalinkova et al., 2003). These studies suggest that ALT could be a common mechanism of telomere length maintenance in angiosarcoma. However, this possibility remains to be confirmed by demonstration of the specific molecular characteristics of ALT-immortalized cells, such as very long heterogeneous telomere lengths, DNA c-circles and PML bodies in angiosarcoma tissue (Bryan et al., 1997; Henson et al., 2005; Fasching et al., 2007).

**Tumorigenic conversion of human EC lines**

Immortalized EC lines established by co-expression of hTERT and SV40 T antigen exhibit a partially transformed phenotype (Table 4) (Salmon et al., 2000; O’Hare et al., 2001b; MacKenzie et al., 2002; Krump-Konvalinkova et al., 2003). BMECs that co-expressed SV40 T antigen and hTERT were shown to be capable of robust anchorage-independent growth in soft-agarose, but were not overtly tumorigenic, because implantation in immune-compromised mice resulted in very small subcutaneous nodules that regressed within 3 weeks (MacKenzie et al., 2002). Pulmonary and sinusoidal ECs transformed with hTERT and SV40 ER were similarly non-tumorigenic (Salmon et al., 2000; Krump-Konvalinkova et al., 2001). In addition to the downregulation of phenotypic markers, ECs immortalized by SV40 T antigen grew independently of exogenous VEGF (Tables 2, 4), which is consistent with the constitutive activation of VEGF signaling and heterogeneous phenotype observed in human vascular neoplasias (Ohsawa et al., 1995; MacKenzie et al., 2002; Bais et al., 2003; Itakura et al., 2008; Young et al., 2010).

Overexpression of oncogenic N-Ras mediated tumorigenic conversion of immortalized BMECs (MacKenzie et al., 2002). Upon subcutaneous injection into immunocompromised mice, BMECs that co-expressed hTERT, SV40 T antigen and N-Ras generated large, rapidly growing tumors that were organized into lumen-like structures and in some mice metastasized to lymph nodes (MacKenzie et al., 2002). When injected via the tail vein, the ECs lodged within the lungs, where they formed tumors that caused morbidity within 3 weeks of implantation. Similarly, human umbilical vein endothelial cells (HUVECs) transfected with HPV E6 and E7 oncogenes and immortalized via spontaneous activation of telomerase were vulnerable to tumorigenic conversion by transfection with activated K-ras (Rhim et al., 1998). The demonstration of K-ras mutations in angiosarcoma underscores the relevance of these models to human vascular neoplasias (Bovin-Angèle et al., 2000; Hong et al., 2000).

**EC hybrid and vascular tumor-derived cell lines**

In addition to genetically engineered ECs, immortalized EC lines have been generated by fusion of HUVECs with human tumor cell lines, such as the adenocarcinoma A549 cell line (Table 5) (Edgell et al., 1983; Hohenwarter et al., 1992a; Unger et al., 2002b). Hybrid endothelial tumor cell lines have unlimited replicative potential and retain some, but not all, EC characteristics (Unger et al., 2002a). In addition, because the hybrid EC A549 cell line displays karyotypic changes that have also been identified in A549 cells, it does not seem an ideal representation of the molecular biology of endothelial tumor cells (Edgell et al., 1983).

Authentic EC lines isolated directly from vascular tumors are clearly valuable for studies of the molecular biology of the disease. Cell lines established by ex vivo culture of human tumor tissues include the angiosarcoma-derived cell lines ISO-HAS, AS-M and HAEND (Hoover et al., 1993; Masuzawa et al., 1999; Unger et al., 2002a; Krump-Konvalinkova et al., 2003). Aberrant p53 expression, a lack of telomerase activity and an ability to form tumors in vivo (evaluated in ISO-HAS and ISO-HAS.1) are some of the characteristics that angiosarcoma-derived clonal EC lines have in common with primary angiosarcomas. At least four KS-derived EC lines have been established from AIDS- and non-AIDS-associated KS patients (Table 5) (Salahuddin et al., 1988; Lunardi-Iskandar et al., 1995b; Popescu et al., 1990; Albini et al., 1997; Casalone et al., 2001). Most of the reported EC-tumor-derived cell lines retained typical endothelial characteristics, including expression of CD31, VEGFR1, vWF and CD1-CAM. One exception was the HAEND cell line, which suppressed expression of all of
## Table 4. EC lines transformed with multiple exogenous genes

| EC phenotype | Tissue source | Cell line name | Life span | Anchorage independent | Transformation | Lifespana | Karyotypeb | References |
|--------------|---------------|----------------|-----------|-----------------------|---------------|-----------|-----------|------------|
| CD31−VEGFR1− | Bone marrow   | BMSVThTERT     | −         | −                     | Simple        | −         | −         | MacKenzie et al., 2002 |
|              | Umbilical vein| E6-E7 HUVEC    | 2G        | +                     | +             | +/−       | NE        |印花等人，2002 |
|              | Umbilical vein| 1/8V, 1/64V    | +         | +                     | +             | +         | −         | Gagnon et al., 2002 |
|              | Liver sinusoidal | LSEC         | +         | +                     | +             | −         | −         | MacKenzie et al., 2002 |

Characteristics of the EC phenotype, i.e. the direction of the expression change for each of the molecular markers, is explained in the text. A positive sign indicates correspondence with the EC phenotype, a negative sign indicates deviation from the EC phenotype.

A simple karyotype is characterized by a near-diploid chromosome number and two or fewer karyotypic abnormalities. A complex karyotype included five or more chromosomal aberrations. An unstable karyotype is characterized by a rapidly evolving karyotype with a high frequency of random aberrations and numerous non-clonal marker and ring chromosomes.

Anchorage-independent growth was determined by in vitro culturing of cells in soft agarose. Tumor formation following subcutaneous injection of cells in NOD/SCID or Balb/c nude mice. Gene was introduced at a later time point. Immortalized via growth crisis.

EC, endothelial cell; NE, not evaluated.

### Animal models of vascular neoplasias

In addition to cell lines, animal models are required for preclinical testing of novel therapeutics. Models that have been established for in vivo study of vascular neoplasias include mice and non-human primates treated with chemical carcinogens or infected with KSHV. Whereas infection with purified KSHV provides a relevant model of KS (Dittmer et al., 1999; Mutlu et al., 2007; Chang et al., 2009), the high incidence of Ras mutations in chemically induced murine vascular neoplasia is consistent with human angiosarcoma (Hong et al., 1980; Sato et al., 1986). Other murine models include mice with a combined knock out of TP53 and the gene encoding p18INK4c, which results in hepatic angiosarcoma with a very high penetrance (Dill et al., 2012). However, to date, Notch1 defects have not been demonstrated in human angiosarcoma.

Clinical trials of novel therapeutics are usually preceded by in vivo tests using patient-derived cell lines or genetically engineered cell lines xenografted in animal models. Tumors that form in mice xenografted with human ECs co-expressing viral antigens that inactivate tumor suppressors p53 and pRB, together with exogenous expression of hTERT and oncogenic Ras, could be used to model angiosarcoma or malignant hemangiendothelioma (Table 4) (Rhim et al., 1998; MacKenzie et al., 2002). By contrast, cavernous endothelial tumors established by infection...
of neonatal mice with polyoma virus middle T antigen, which induces constitutive tyrosine kinase activity, might be a more relevant model of infantile hemangioma (Bautch et al., 1987). A benign neoplasia resembling hemangioma was also modeled in mice xenografted with HUVECs expressing polyoma virus middle T antigen. The xenografted cells formed tumors that regressed after 3 weeks, mirroring the typical course of hemangioma (Primo et al., 2000; Schaffhausen and Roberts, 2009).

Engraftment of patient-derived cells in immunocompromised mice is a clinically relevant alternative to genetically engineered xenograft models. Similar to the polyoma middle T-antigen–driven xenograft tumors, patient-derived hemangioma progenitor cells were shown to form transient subcutaneous tumors when engrafted in immunocompromised mice (Khan et al., 2008; Xu et al., 2011). Xenograft tumors have also been established from canine angiosarcoma (Akhtar et al., 2004; Kodama 2011). Xenograft tumors have also been established from canine angiosarcoma (Akhtar et al., 2004; Kodama et al., 2009). In addition to providing a useful model for preclinical testing of novel therapeutics, xenografts potentially provide a means for expansion of rare tumor material via serial transplantation (Khan et al., 2008).

**Perspectives and conclusions**

Thus far, investigations of the molecular biology of EC-derived malignancies have been limited to molecular and cytogenetic case studies and investigations of specific oncogenes and tumor suppressors in small patient cohorts. The data accumulated to date implicate abnormalities in the VEGF pathway as a common event across the spectrum of benign and malignant vascular neoplasms (Fig. 1). Defects in the TP53 and p16INK4a–RB tumor suppressor pathways feature in intermediate and aggressive malignancies, and are often associated with the development of chromosomal abnormalities. It is notable that, aside from aberrant VEGF signaling, there have been no reports of oncogenic changes that clearly distinguish EC neoplasias from other cancers, such as epithelial cancers. Hence, genetic models of EC neoplasias have largely relied on combinations that have also been shown to transform epithelial cells (Boehm and Hahn, 2005).

Studies involving the genetic manipulation of human ECs have confirmed that perturbation of TP53 and p16INK4a–RB tumor suppressor pathways, together with the activation of a telomere maintenance mechanism, are crucial for EC immortalization. However, in contrast to carcinomas, which predominantly rely on telomerase for telomere maintenance, the limited literature available suggests that the activation of ALT might be relatively common in angiosarcoma. Nevertheless, ALT-immortalized EC lines are underrepresented among the currently available EC lines. In contrast to the well-documented approach to the reconstitution of telomerase by overexpression of hTERT, there are currently no known means of directly activating ALT in primary mammalian cells. However, studies that showed...
spontaneous activation of ALT in SV40-transformed epithelial and fibroblastic cell lines allude to an indirect means of establishing ALT-immortalized EC lines (Bryan et al., 1995).

The currently available genetic models might also be made more clinically relevant by substituting viral oncocenes with short hairpin RNA (shRNA) targeting the specific tumor suppressor genes implicated in EC malignancies, and by constitutive activation of VEGF signaling. Conditional deletion of tumor suppressor genes specifically within ECs would also provide a valuable alternative murine model of endothelial neoplasia. Additional refinement of genetic models will then be contingent upon further characterization of the specific molecular defects that underpin the initiation and progression of specific vascular neoplasias. Although hampered by sample numbers, genome-wide mapping and next-generation sequencing might be applied toward this goal. Information from broad-platform technologies such as these will be valuable for improving diagnosis, identifying therapeutic targets, and establishing new in vitro and in vivo models that accurately depict the molecular biology of specific EC-derived neoplasias. Such models will then provide an avenue for preclinical tests of new therapeutics and for ultimately improving outcomes for patients with these disorders.

COMPETING INTERESTS

The authors declare that they do not have any competing or financial interests.

FUNDING

The Children's Cancer Institute Australia is affiliated with the University of New South Wales and the Sydney Children's Hospital Network. K.L.M. received research grants from National Health and Medical Research Council Career Development Award (510378); NSW Cancer Council and Cancer Institute NSW.

REFERENCES

Baur, V. L., Toda, S., Hassell, J. A. and Hanahan, D. (1987). Endothelial cell tumors develop in transgenic mice carrying polyoma virus middle T oncogene. Cell 51, 529-537.

Berg, J. N., Walter, J. W., Thisanagayam, U., Evans, M., Bief, F., Waner, M., Diamond, A. G., Marchuk, D. A. and Porteous, M. E. (2001). Evidence for loss of heterozygosity of 5q in sporadic haemangiomata: are somatic mutations involved in haemangioma formation? J. Clin. Pathol. 54, 249-252.

Boehm, J. S. and Hahn, W. C. (2005). Cancer genetics: Finding the right mix. Eur. J. Hum. Genet. 13, 1099-1100.

Boivin-Angélie, S., Lefrançois, L., Froment, O., Spiethoff, A., Bogdannfy, M. S., Wegeninger, K., Wesch, H., Barbin, A., Bancel, B., Trépo, C. et al. (2000). Ras gene mutations in vinyl chloride-induced liver tumors are carcinogenic-specific but vary with cell type and species. Int. J. Cancer 85, 223-227.

Boshoff, C., Schulz, T. F., Kennedy, M. M., Graham, A. K., Fisher, C., Thomas, A., McGee, J. O., Weiss, R. A. and O'Leary, J. J. (1995). Kaposi's sarcoma-associated herpesvirus infects endothelial and spindle cells. Nat. Med. 1, 1274-1278.

Boyce, E., Yu, Y., Paranya, G., Mulliken, J. B., Olsen, B. R. and Bishop, J. (2001). Clonality and altered behavior of endothelial cells from hemangiomas. J. Clin. Invest. 107, 745-752.

Brooks, J. J. (1986). Kaposi's sarcoma: a reversible hyperplasia. Lancet 328, 1309-1311.

Bryan, T. M., Engle louz, A., Gupta, J., Bacchetti, S. and Reddel, R. R. (1995). Telomere elongation in immortal human cells without detectable telomerase activity. EMBO J. 14, 4240-4248.

Bryan, T. M., Engle louz, A., Del Pozo, L., Dunham, M. A. and Reddel, R. R. (1997). Evidence for an alternative mechanism for maintaining telomere length in immortalized human cells and tumors-derived cell lines. Nat. Med. 3, 1271-1274.

Burger, A. M., Fiebig, H. H., Kuetttel, M. R., Lautenberger, J. A., Kung, H. F. and Rhim, J. S. (1998). Effect of oncogene expression on telomerase activation and telomere length in human endothelial, fibroblast and prostate epithelial cells. Int. J. Oncol. 13, 1043-1048.

Candolf, F. J., Rafii, S., Parker, J. T., Ades, E. W., Ferris, B., Ranach, N. M. and Kellar, K. L. (1996). BMEC-1: a human bone marrow microvascular endothelial cell line with primary cell characteristics. Microvasc. Res. 52, 221-234.

Carmeliet, P. and Jain, R. K. (2011). Molecular mechanisms and clinical applications of angiogenesis. Nature 473, 298-307.

Casalono, R., Albinzi, A., Righi, R., Granata, P. and Toniolo, A. (2001). Nonrandom chromosome changes in Kaposi sarcoma: cytogenetic and FISH results in a new cell line (KS-IMM) and literature review. Cancer Genet. Cytogenet. 124, 16-19.

CeriIlli, L. A., Huffman, H. T. and Andan, A. (1998). Primary renal angiosarcoma: a case report with immunohistochemical, ultrastructural, and cytogenetic features and review of the literature. Arch. Pathol. Lab. Med. 122, 929-935.

Chandler, H. L., Newkirk, K. M., Kusewitt, D. F., Dubielzig, R. R. and Colitz, C. M. (2009). Immunohistochemical analysis of ocular hemangiomas and hemangiosarcomas in dogs. Vet. Ophthalmol. 12, 83-90.

Chang, E. and Harley, C. B. (1995). Telomere length and replicative aging in human vascular tissues. Proc. Natl. Acad. Sci. USA 92, 11190-11194.

Chang, Y., Cesarman, E., Pessin, M. S., Lee, F., Culpepper, J., Knowles, D. M. and Moore, P. S. (1994). Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi’s sarcoma. Science 266, 1865-1869.

Chang, H., Wachtman, L. M., Pearson, C. B., Lee, J. S., Lee, H. R., Lee, S. H., Vieira, J., Mansfield, K. G. and Jung, J. U. (2009). Non-human primate model of Kaposi's sarcoma-associated herpesvirus infection. PLoS Pathog. 5, e1000606.

Chen, Z., Smith, K. J., Skelton, H. G., 3rd, Barrett, T. L., Greenway, H. T., Jr and Lo, S. C. (2001). Telomerase activity in Kaposi's sarcoma, squamous cell carcinoma, and basal cell carcinoma. Exp. Biol. Med. (Maywood) 226, 753-757.

Cohen, S. B., Graham, M. E., Lovrecz, G. O., Bache, N., Robinson, P. J. and Reddel, R. R. (2007). Protein composition of catalytically active human telomerase from immortal human cells. Science 315, 1850-1853.

Counter, C. M., Avilion, A. A., LeFeuvre, C. E., Stewart, N. G., Greider, C. W., Harley, C. B. and Bacchetti, S. (1992). Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. EMBO J. 11, 2921-2929.

Daldras, S. S., North, P. E., Bertoncini, J., Mihm, M. C. and Detmar, M. (2004). Infantile hemangiomas are arrested in an early developmental vascular differentiation state. Mod. Pathol. 17, 1068-1079.

Dill, M. T., Rothweiler, S., Djovon, V., Hlushchuk, R., Tomillo, L., Terracciano, L., Meili-Butz, S., Radtke, F., Heim, M. H. and Semela, D. (2012). Disruption of Notch1 induces vascular remodeling, intussusceptive angiogenesis, and angiosarcomas in mice of liver. gastroent. 142, 967-977, e2.

Dimri, G. P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E. E., Linskens, M., Rubelj, I., Pereira-Smith, O. et al. (1995). A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc. Natl. Acad. Sci. USA 92, 9363-9367.
Dittmer, D., Stoddart, C., Renne, R., Linquist-Stepps, V., Moreno, M. E., Bare, C., McCune, J. M. and Ganem, D. (1999). Experimental transmission of Kaposi’s sarcoma-associated herpesvirus (KSHV/HHV-8) to SCID-hu Th/Liv mice. J. Exp. Med. 190, 1857-1868.

Domfeh, A. B., Fichera, M. and Hunt, J. L. (2006). Allole loss of 3 different tumor suppressor gene loci in benign and malignant endothelial tumors of the head and neck. Arch. Pathol. Lab. Med. 130, 1184-1187.

Drolet, B. A., Esterly, N. B. and Frieden, I. J. (1999). Hemangiomias in children. N. Engl. J. Med. 341, 173-181.

Ducray, C., Pommier, J. P., Martins, L., Bousfin, D. F. and Sabatier, L. (1999). Telomere dynamics, end-to-end fusions and telomerase activation during the human fibroblast immortalization process. Oncogene 18, 4211-4223.

Edgell, C. J., McDonald, C. C. and Graham, J. B. (1983). Permanent cell line expressing human factor VIII-related antigen established by hybridization. Proc. Natl. Acad. Sci. USA 80, 3734-3737.

Fasching, C. L., Neumann, A. A., Muntoni, A., Yeager, T. R. and Reddel, R. R. (2007). DNA damage induces alternative lengthening of telomeres (ALT) associated promyelocytic leukemia bodies that preferentially associate with linear telomeric DNA. Cancer Res. 67, 7072-7077.

Feng, J., Funk, W. D., Wang, S.-S., Weinrich, S. L., Avillon, A. A., Chiu, C.-P., Adams, R. R., Chang, E., Allsopp, R. C., Yu, J. et al. (1995). The RNA component of human telomerase. Science 269, 1236-1241.

Fickling, S. A., Tooze, J. A. and Whiteley, G. S. (1992). Characterization of human umbilical vein endothelial cell lines produced by transfection with the early region of SV40. Exp. Cell Res. 201, 517-521.

Fontijn, R., Hop, C., Brinkman, H. J., Slater, R., Westerveld, a, van Mourik, J. A. and Pannekoek, H. (1995). Maintenance of vascular endothelial cell-specific properties after immortalization with an amphotrophic replication-deficient retrovirus containing human papilloma virus 16 E6/E7 DNA. Exp. Cell Res. 216, 199-207.

Freedman, D. A. (2005). Senescence and its bypass in the vascular endothelium. Front. Biosci. 10, 940-950.

Freedman, D. A. and Folkman, J. (2004). Maintenance of G1 checkpoint controls in telomerase-immortalized endothelial cells. Cell Cycle 3, 811-816.

Friborg, J., Jr, Kong, W., Hottiger, M. O. and Nabel, G. J. (1999). p53 inhibition by the LANA protein of KSHV protects against cell death. Nature 402, 889-894.

Gagnon, E., Cattaruzzi, P., Griffith, M., Muzakare, L., LeFiao, K., Faure, R., Béliveau, R., Hussain, S. N., Koutsilieris, M. and Doillon, C. J. (2005). Senescence and its bypass in the vascular endothelium. Front. Biosci. 10, 349-356.

Ganem, D. (2010). KSHV and the pathogenesis of Kaposi sarcoma: listening to human biology and medicine. J. Clin. Invest. 120, 939-949.

Garcia, J. M., Gonzalez, R., Silva, J. M., Dominguez, G., Vegazo, I. S., Gamallo, C., Provencio, M., España, P. and Bonilla, F. (1994). Maintenance of vascular endothelial cell-specific properties after immortalization with an amphotrophic replication-deficient retrovirus containing human papilloma virus 16 E6/E7 DNA. Exp. Cell Res. 216, 199-207.

Ganem, D. (2010). KSHV and the pathogenesis of Kaposi sarcoma: listening to human biology and medicine. J. Clin. Invest. 120, 939-949.

George, S., Merriam, P., Mak, R. G., Van den Abbeele, A. D., Yap, J. T., Akhurst, T., Harmon, D. C., Bhuchar, O., O’Mara, M. M., D’Adamo, D. R. et al. (2009). Multicenter phase II trial of sunitinib in the treatment of nongastrointestinal stromal tumour sarcomas. J. Clin. Oncol. 27, 3154-3160.

Gil-Benso, R., López-Ginés, C., Soriano, P., Almenar, S., Vazquez, C. and Llombart-Bosch, A. (1994). Cyto genetic study of angiosarcoma of the breast. Genes Chromosomes Cancer 10, 210-212.

Gimbrone, M. A., Jr and Fareed, G. C. (1976). Transformation of cultured human vascular endothelium by SV40 DNA. Cell 9, 685-693.

Girardi, A. J., Jensen, F. C. and Koprowski, H. (1983). Permanent cell line derived from a Kaposi sarcoma sarcomas. Proc. Natl. Acad. Sci. USA 80, 6769-6773.

Girardi, A. J., Jensen, F. C. and Koprowski, H. (1983). Permanent cell line derived from a Kaposi sarcoma sarcomas. Proc. Natl. Acad. Sci. USA 80, 6769-6773.

Girardi, A. J., Jensen, F. C. and Koprowski, H. (1983). Permanent cell line derived from a Kaposi sarcoma sarcomas. Proc. Natl. Acad. Sci. USA 80, 6769-6773.

Girardi, A. J., Jensen, F. C. and Koprowski, H. (1983). Permanent cell line derived from a Kaposi sarcoma sarcomas. Proc. Natl. Acad. Sci. USA 80, 6769-6773.

Girardi, A. J., Jensen, F. C. and Koprowski, H. (1983). Permanent cell line derived from a Kaposi sarcoma sarcomas. Proc. Natl. Acad. Sci. USA 80, 6769-6773.

Girardi, A. J., Jensen, F. C. and Koprowski, H. (1983). Permanent cell line derived from a Kaposi sarcoma sarcomas. Proc. Natl. Acad. Sci. USA 80, 6769-6773.

Girardi, A. J., Jensen, F. C. and Koprowski, H. (1983). Permanent cell line derived from a Kaposi sarcoma sarcomas. Proc. Natl. Acad. Sci. USA 80, 6769-6773.

Girardi, A. J., Jensen, F. C. and Koprowski, H. (1983). Permanent cell line derived from a Kaposi sarcoma sarcomas. Proc. Natl. Acad. Sci. USA 80, 6769-6773.

Girardi, A. J., Jensen, F. C. and Koprowski, H. (1983). Permanent cell line derived from a Kaposi sarcoma sarcomas. Proc. Natl. Acad. Sci. USA 80, 6769-6773.
Koch, M., Nielsen, G. P. and Yoon, S. S. (2008). Malignant tumors of blood vessels: angiosarcomas, hemangioendothelomas, and hemangiopericytomas. J. Surg. Oncol. 97, 321-329.

Kodama, A., Sakai, H., Matsuura, S., Murakami, M., Murai, A., Mori, T., Maruo, K., Kimura, T., Masugi, M. and Yanai, T. (2009). Establishment of canine hemangiosarcoma xenograft models expressing endothelial growth factor receptors, their receptors, and angiogenesis-associated homeobox genes. BMC Cancer 9, 363.

Koon, H. B., Bubley, G. J., Pantanowitz, L., Masiello, D., Smith, B., Crosby, K., Proper, J., Weeden, W., Miller, T. E., Chats, P. et al. (2005). Imatinib-induced regression of AIDS-related Kaposi's sarcoma. J. Clin. Oncol. 23, 982-989.

Koontz, B. F., Miles, E. F., Rubio, M. A., Madden, J. F., Fisher, S. R., Scher, R. L. and Bristz, D. M. (2008). Preoperative radiotherapy and bevacizumab for the treatment of head and neck cancer: a two case studies. Head Neck 30, 262-266.

Krishnaswamy, G., Kelley, J., Yerra, L., Smith, J. K. and Chi, D. S. (1999). Human endothelium as a source of multifunctional cytokines: molecular regulation and possible role in human disease. J. Interferon Cytokine Res. 19, 91-104.

Krump-Konvalinkova, V., Bittinger, F., Unger, R. E., Peters, K., Lehr, H. A. and Krishnaswamy, G., Kelley, J., YerrA, L., Smith, J. K. and Chi, D. S. (1999). Hemangiosarcoma cell line (ISO-HAS).

Mandahl, N., Jin, Y. S., Heim, S., Willén, H., Wennerberg, J., Biörklund, A. and Maki, R. G., D’Adamo, D. R., Keohan, M. L., Saulle, M., Schuetze, S. M., Undevia, S. et al. (2000). Establishment of canine hemangiosarcoma cell line (ISO-HAS).

Moore, M. A. (1995b). Isolation and characterization of an immortal neoplastic cell line (KS Y-1) from AIDS-associated Kaposi's sarcoma.

Nichols, W. W., Buynak, E. B., Bradt, C., Hill, R., Aronson, M., Jarrell, B. E., Mueller, S. N. and Levine, E. M. (1987). Cytogenetic evaluation of human endothelial cell cultures. J. Cell Physiol. 132, 465-462.

Nisato, R. E., Harrison, J. A., Buser, R., Orci, L., Rincón, C., Montesano, R., Dupraz, P. and Pepper, M. S. (2004). Generation and characterization of telomerase-negative lymphatic endothelial cells with an extended life span. Am. J. Pathol. 165, 11-24.

O’Hagan, R. C., Chang, S., Maser, R. S., Mohan, R., Artandi, S. E., Chin, L. and DePinho, R. A. (2003). Telomere dysfunction provokes regional amplification and deletion in cancer genomes. Cancer Cell 2, 149-155.

O’Hare, M. J., Bond, J., Clarke, C., Takeuchi, Y., Atherton, A. J., Berry, C., Moody, J., Iskandar, Y. M., Davies, D. C., Alsop, A. E. et al. (2001). Conditional immortalization of freshly isolated human mammary fibroblasts and endothelial cells. Proc. Natl. Acad. Sci. USA 98, 646-651.

Oshawa, M., Naka, N., Tomita, Y., Kawamori, D., Kanno, H. and Aozasa, K. (1995). Use of immunohistochemical procedures in diagnosing angiosarcoma. Evaluation of 98 cases. Cancer 75, 2867-2874.

Okuda, K., Khan, M. Y., Skurnick, J., Kimura, M., Aviv, H. and Aviv, A. (2000). Telomere attrition of the human abdominal aorta: relationships with age and atherosclerosis. Atherosclerosis 152, 391-398.

Pareja, M. J., Vargas, M. T., Sánchez, A., Ibáñez, J. and González-Cámpora, R. (2009). Cytogenetic study of a pulmonary sclerosing hemangioma. Cancer Genet. Cytogenet. 195, 80-84.

Popescu, N. C., Zimonjic, D. B., Leventon-Kriss, S., Bryant, J. L., Lunardi-Iskandar, Y. and Gallo, R. C. (1996). Deletion and translocation involving chromosome 3 (p14) in two tumorigenic Kaposi’s sarcoma cell lines. J. Natl. Cancer Inst. 88, 450-455.

Pyakurel, P., Pak, F., Mwakigonja, A. R., Kaaya, E., Heiden, T. and Biberfeld, P. (2006). Lymphatic and vascular origin of Kaposi's sarcoma spindle cells during tumor development. Int. J. Cancer 119, 1262-1267.

Ray, F. A., Peabody, D. S., Cooper, J. L., Cram, L. S. and Kraemer, P. M. (1997). Sporadic and Thorotrast-induced angiosarcomas of the liver manifest frequent and multiple point mutations in K-ras-2. Lab. Invest. 76, 153-159.

Reddell, R. R. (2000). The role of senescence and immortalization in carcinogenesis. Carcinogenesis 21, 477-484.

Rhim, J. S., Tsai, W. P., Chen, Z. Q., Chen, Z., Van Waes, C., Burger, A. M. and Lautenberger, J. A. (1999). A human vascular endothelial cell model to study angiogenesis and tumorigenesis. Carcinogenesis 19, 673-681.

Ribeiro, M. J., Phillips, D. J., Benson, J. M., Evatt, B. L., Ades, E. W. and Hooper, W. C. (1995). Hemostatic properties of the SV-40 transfected human microvascular endothelial cell line (HMEC-1). A representative in vitro model for microvascular endothelium. Thromb. Res. 79, 153-161.

Rudolph, K. L., Millard, M., Bosenberg, M. W. and DePinho, R. A. (2001). Telomere dysfunction and evolution of intestinal carcinoma in mice and humans. Nat. Genet. 28, 155-159.

Salahuddin, S. Z., Nakamura, S., Biberfeld, P., Kaplan, M. H., Markham, P. D., Larsson, L. and Gallo, R. C. (1988). Angiogenic properties of Kaposi’s sarcoma-derived cells after long-term culture in vitro. Science 242, 430-433.

Salmon, P., Oberholzer, J., Ochiodoro, T., Morel, P., Lou, J. and Tronco, D. (2000). Reversible immortalization of human primary cells by lentivirus-mediated transfer of specific genes. Mol. Ther. 2, 404-414.
Sato, N., Sato, T., Takahashi, S. and Kikuchi, K. (1986). Establishment of murine endothelial cell lines that develop angiosarcomas in vivo: brief demonstration of a proposed animal model for Kaposis’s sarcoma. Cancer Res. 46, 362-366.

Schauffhausen, B. S. and Roberts, T. M. (2009). Lessons from polymia middle T antigen on signaling and transformation: A DNA tumor virus contribution to the war on cancer. Virology 384, 304-316.

Schuborg, C., Mertens, F., Rydholm, A., Brosjö, O., Dictor, M., Mitelman, F. and Mandahl, N. (1998). Cytogenetic analysis of four angiosarcomas from deep and superficial soft tissue. Cancer Genet. Cytogenet. 100, 52-56.

Schwartz, B., Vicart, P., Delouis, C. and Paulin, D. (1991). Mammalian cell lines can be efficiently established in vitro upon expression of the SV40 large T antigen driven by a promoter sequence derived from the human vimentin gene. Biol. Cell. 73, 7-14.

Shao, R. and Guo, X. (2004). Human microvascular endothelial cells immortalized with human telomerase catalytic protein: a model for the study of in vitro angiogenesis. Biochem. Biophys. Res. Commun. 321, 788-794.

Shay, J. W., Pereira-Smith, O. M. and Wright, W. E. (1991). A role for both RB and p53 in the regulation of human cellular senescence. Exp. Cell Res. 196, 33-39.

Shi, Q., Raffi, S., Wu, M. H., Wijelath, E. S., Yu, C., Ishida, A., Fujita, Y., Kothari, S., Mohle, R., Sauvage, L. R. et al. (1998). Evidence for circulating bone marrow-derived endothelial cells. Blood 92, 362-367.

Sie, H. and Robertson, E. S. (2000). Kaposis sarcoma-associated herpesvirus-encoded latency-associated nuclear antigen induces chromosomal instability through inhibition of p53 function. J. Virol. 80, 697-709.

Sodhi, A., Chaisuparat, R., Hu, J., Ramsdell, A. K., Manning, B. D., Sausville, E. A., Sawai, E. T., Molinolo, A., Gutkind, J. S. and Montaner, S. (2006). The TSC2/mTOR pathway drives endothelial cell transformation induced by the Kaposis sarcoma-associated herpesvirus G protein-coupled receptor. Cancer Cell 10, 133-143.

Stein, G. H., Drullinger, L. F., Soulard, A. and Dulić, V. (1994). Cellular markers that distinguish the phases of hemangioma during infancy and childhood. J. Clin. Invest. 93, 2357-2364.

Sow, K. F., So, C. C., Wong, N., Siu, L. L., Kwong, Y. L. and Chan, J. K. (2009). Lessons from polyoma middle T antigen on signaling and transformation: A DNA tumor virus contribution to the war on cancer. Virology 384, 304-316.

Takano, H., Hirasawa, S. and Asahara, T. (2002a). In vitro expression of the endothelial phenotype: comparative study of primary angiogenic cells and cell lines, including the novel cell line HPMEC-ST1.6R. J. Vet. Med. Sci. 64, 357-364.

Takano, H., Murasawa, S. and Asahara, T. (2008). Functional and gene expression analysis of hTERT overexpressed endothelial cells. Biology 2, 547-554.

Tambruni, B. A., Trapp, S., Phang, T. L., Schappa, J. T., Hunter, L. E. and Modiano, J. F. (2009). Gene expression profiles of sporadic canine hemangiosarcoma are uniquely associated with breed. PLOS ONE 4, e5549.

Tannapfel, A., Weihrauch, M., Benicke, M., Uhlmann, D., Hauss, J., Wrbitzky, R. and Wittdeck, C. (2001). p16INK4A alterations in primary angiosarcoma of the liver. J. Hepatol. 35, 62-67.

Theuillat, J. P., Vavraka, S. R., Weishaupt, D., Dictor, M., Mitelman, F. and Mandahl, N. (1998). Cytogenetic analysis of four angiosarcomas from deep and superficial soft tissue. Cancer Genet. Cytogenet. 100, 52-56.

Weihrauch, M., Markworth, A., Lehnert, G., Wittekind, C., Wrbitzky, R. and Tannapfel, A. (2002). Abnormalities of the ARF-p53 pathway in primary angiosarcomas of the liver. Hum. Pathol. 33, 884-892.

Wen, V. W., Wu, K., Baksh, S., Hanselwood, R. A., Lock, R. B., Clark, S. J., Moore, M. A. and Mackenzie, K. L. (2006). Telomere-driven karyotypic complexity concurs with p16INK4a inactivation in TP53-competent immortal endothelial cells. Cancer Res. 66, 10691-10700.

Werners, B. A., Levine, A. J. and Howley, P. M. (1990). Association of human papillomavirus types 16 and 18 with proteins in p53. Science 248, 76-79.

Wong, K. F., So, C. C., Wong, N., Siu, L. L., Kwong, Y. L. and Chan, J. K. (2001). Sinonasal angiosarcoma with marrow involvement at presentation mimicking malignant lymphoma: cytogenic analysis using multiple techniques. Cancer Genet. Cytogenet. 129, 64-68.

Wright, W. E. and Fratyszczek, M. A., Rainey, W. E., Byrd, W. and Shay, J. W. (1996). Telomerase activity in human germline and embryonic tissues and cells. Dev. Genet. 17, 173-196.

Xu, D., Neville, R. and Finkel, T. (2000). Homocystine accelerates endothelial cell senescence. FEBS Lett. 470, 20-24.

Xu, D. O., T. M., Shartava, A., Fowellies, T. C., Yang, J., Fink, L. M., Ward, D. C., Milmh, M. C., Wener, M. and Ma, Y. (2011). Isolation, characterization, and in vitro propagation of infantile hemangioendothelioma stem cells and an in vivo mouse model. Hematol. Oncol. 4, 54.

Yang, J., Chang, E., Cherry, A. M., Bangs, C. D., Oei, Y., Bodnar, A., Bronstein, A., Chiu, C. P. and Herron, G. S. (1999). Human endothelial cell life extension by telomerase expression. J. Biol. Chem. 274, 26141-26148.

Yang, J., Nagavarapu, U., Hellima, K., Sjastad, M. D., Moss, W. C., Passaniti, A. and Herron, G. S. (2001). Telomerized human microvasculature is functional in vivo. Nat. Biotechnol. 19, 219-224.

Yazawa, M., Okuda, M., Setoguchis, A., Nishimura, R., Sasaki, N., Hasegawa, A., Watarai, T. and Tsujimoto, H. (1999). Measurement of telomerase activity in dog tumors. J. Vet. Med. Sci. 61, 1125-1129.

Yonekura, K., Sakai, H., Murakami, M., Kodama, A., Mori, T., Yanai, T., Maruo, K. and Masegi, T. (2007). The significance of p53 and retinoblastoma pathways in canine hemangiosarcoma. J. Vet. Med. Sci. 69, 271-278.

Young, A. T., Lakey, J. R., Murray, A. G., Mullin, J. C. and Moore, R. B. (2003). In vitro senescence occurring in normal human endothelial cells can be rescued by ectopic telomerase activity. Transplant. Proc. 35, 2483-2485.

Young, R. J., Brown, N. J., Reed, M. W., Hughes, D. and Woll, P. J. (2010). Angiosarcoma. Lancet Oncol. 11, 983-991.

Yuan, J., Wen, V. W., Schuller, C. E. and MacKenzie, K. L. (2008). Molecular mechanisms that regulate the replicative lifespan of endothelial cells. Cell Apoptosis Research Progress (ed. R. H. Fenton and C. V. Burnside), pp. 83-113. New York, NY: Nova Science Publishers.

Zhang, L., Avis, H., Gardner, J. P., Okuda, K., Patel, S., Kirmura, M., Bardgeuz, A. and Avis, A. (2000). Loss of chromosome 13 in cultured human vascular endothelial cells. Exp. Cell Res. 260, 357-364.

Zhou, B. B., Zhang, H., Danmell, M., Geis, K. G., Grindley, J. C. and Dirks, P. B. (1999). Measurement of telomerase activity in dog tumors. J. Vet. Med. Sci. 61, 1125-1129.