REVIEW

Viruses as key regulators of angiogenesis

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SUMMARY

Angiogenesis is an important physiological process that is controlled by a precise balance of growth and inhibitory factors in healthy tissues. However, environmental and genetic factors may disturb this delicate balance, resulting in the development of angiogenic diseases, tumour growth and metastasis. During the past decades, extensive research has led to the identification and characterization of genes, proteins and signalling pathways that are involved in neovascularization. Moreover, increasing evidence indicates that viruses may also regulate angiogenesis either directly, by (i) producing viral chemokines, growth factors and/or receptors or (ii) activating blood vessels as a consequence of endothelial cell tropism, or indirectly, by (iii) modulating the activity of cellular proteins and/or (iv) inducing a local or systemic inflammatory response, thereby creating an angiogenic microenvironment. As such, viruses may modulate several signal transduction pathways involved in angiogenesis leading to changes in endothelial cell proliferation, migration, adhesion, vascular permeability and/or protease production. Here, we will review different mechanisms that may be applied by viruses to deregulate the angiogenic balance in healthy tissues and/or increase the angiogenic potential of tumours. Copyright © 2011 John Wiley & Sons, Ltd.

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ANGIOGENESIS

Angiogenesis involves the formation of new blood vessels from pre-existing vessels. In adults,

neovascularization is limited to wound healing and the female reproductive cycle. Under these physiological conditions, angiogenesis is well-regulated and only switched on for a short period of time. Unregulated angiogenesis may lead to many deadly and debilitating conditions, including cancer, skin diseases, blindness and cardiovascular disease [1].

Blood vessels consist of a layer of endothelial cells (ECs) surrounded by pericytes, which stabilize the vessels and provide survival signals. The first step in the angiogenesis cascade is the release of pro-angiogenic factors in response to hypoxia or nutrient deprivation [2]. Hypoxia-inducible factor-1 (HIF-1) is the principal regulator of angiogenesis. This transcription factor is composed of an oxygen-sensitive α subunit and a constitutively expressed β subunit. Under normoxic conditions, the α subunit is rapidly degraded via a proteasome-degradation pathway (Figure 1). In contrast, under hypoxia, the α subunit is able to accumulate and form a heterodimer with the β subunit. This active transcription factor binds to hypoxia-response elements in the promoter of various genes, whose products are involved in angiogenesis [3]. Next, pro-angiogenic factors [e.g. vascular endothelial growth factor (VEGF), angiopoietins (Ang)] activate the ECs by binding to specific EC receptors

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Abbreviations used:

Ang-2, angiopoietin-2; AP, activator protein; ATLL, adult T-cell leukaemia; BM, basement membrane; CCL2, monocyte chemoattractant protein-1 (MCP-1); CM, conditioned medium; COX-2, cyclooxygenase-2; CXCL1, growth regulated oncogene- α (GRO- α); CXCL4, platelet factor-4 (PF-4); CXCL8, interleukin-8 (IL-8); CXCL12, stromal cell-derived factor-1 (SDF-1); EBNA, EBV nuclear antigen; EC, endothelial cell; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; FGF2, basic fibroblast growth factor; FGFR, fibroblast growth factor receptor; HCC, hepatocellular carcinoma; HIF, hypoxia-inducible factor; HPV, human papilloma virus; HSPG, heparan sulphate-containing proteoglycan; KS, Kaposi's sarcoma; KSHV, Kaposi's sarcoma-associated herpesvirus; LMP, latent membrane protein; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; MT, membrane-type; NF- κ B, nuclear factor- κ B; NHL, non-Hodgkin's lymphoma; NPC, nasopharyngeal carcinoma; PlGF, placenta growth factor; PDGF, platelet-derived growth factor; PI3K, phosphoinositide 3-kinase; pVHL, von Hippel-Lindau protein; STAT, signal transducer and activator of transcription; TP, thymidine phosphorylase; TIMP, tissue inhibitor of metalloproteinase; TVS, transplant vascular sclerosis; VE, vascular endothelial; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; vFLIP, viral FLICE-inhibitory protein; vGPCR, viral G protein-coupled receptor; vIL-6, viral interleukin-6; vIRF, viral interferon regulatory factor.

Figure 1. Viral regulation of HIF-1 activity. Hypoxia-inducible factor-1 (HIF-1), the principal regulator of both physiological and pathological angiogenesis, is composed of an oxygen-sensitive α subunit and a constitutively expressed β subunit. Under normal oxygen conditions, HIF-1 α (blue) is rapidly hydroxylated by prolyl hydroxylases (HIF-PH), which enable the binding of HIF-1 α to the von Hippel-Lindau protein (pVHL, green), a recognition component of the E3 ligase complex. This binding promotes the ubiquitination of HIF-1 α , thereby targeting it for proteasomal degradation. Because prolyl hydroxylases require molecular oxygen for their enzymatic activity, hypoxic conditions inhibit proline hydroxylation of HIF-1 α , resulting in HIF-1 α accumulation and its dimerization with HIF-1 β . The heterodimeric transcription factor binds to hypoxia-response elements (HREs) within the promoter of target genes and activates the expression of a wide variety of genes involved in angiogenesis (e.g. VEGF) (reviewed in [3]). Viruses can interfere with this pathway by increasing *HIF-1 α* transcription and/or protein stability. The viral oncoproteins vGPCR (expressed by KSHV) [65] and HBx (expressed by HBV) [92] up-regulate *HIF-1 α* expression by activating the MAPK pathway. HCV and HTLV-1 have also been shown to increase *HIF-1 α* transcription, although the viral proteins involved have not yet been elucidated [105,166]. HBV HBx stabilizes HIF-1 α in normoxia by inhibiting its interaction with pVHL [91], whereas KSHV LANA stimulates degradation of pVHL, thereby increasing the cellular pool of HIF-1 α [167]. HPV E6 may increase HIF-1 α levels by promoting the degradation of p53. This, in turn, may abolish p53-evoked transcriptional repression of HIF-1 and/or inhibit p53-induced ubiquitination of HIF-1 α [82]. On the other hand, EBV LMP1 up-regulates the E3 ubiquitin ligase, which induces degradation of prolyl hydroxylases that normally mark HIF-1 α for degradation [28]

(see further). As a consequence, the pericytes detach, and the 'activated' ECs produce proteolytic enzymes [e.g. matrix metalloproteinases (MMPs)] that degrade the basement membrane and extracellular matrix (ECM) surrounding the existing vessel [4]. This enables the ECs to migrate and proliferate in the perivascular space in the direction of the source of the angiogenic stimulus (e.g. tumour, hypoxic or inflamed tissue). The newly formed sprout synthesizes a new basement membrane, recruits pericytes and connects with another sprout to allow blood flow through the new capillary [5,6]. These different steps are regulated by a multitude of pro-angiogenic and anti-angiogenic proteins, including soluble factors, ECM components, adhesion molecules and cell-surface receptors (Figure 2).

Soluble factors with angiogenic activity can be largely divided into two groups. The first group

consists of the VEGF family and the angiopoietins, which mainly activate ECs. VEGF, one of the most potent and best characterized angiogenic factors, stimulates EC proliferation and migration, MMP production as well as vascular permeability by activating high-affinity tyrosine kinase receptors VEGFR-1 (Flt1) and VEGFR-2 (KDR/Flk1) on the surface of ECs [7]. Angiopoietins (Ang-1 and Ang-2) are ligands of the endothelial-specific tyrosine kinase receptor Tie-2. The binding of Ang-1 to Tie-2 results in receptor activation and the transduction of signals that promote blood vessel stabilization. In contrast, Ang-2, by inhibiting the interaction of Ang-1 with Tie-2, induces destabilization of the vessels, rendering them more responsive to angiogenic factors, such as VEGF [8].

The second class consists of growth factors, chemokines, cytokines and angiogenic enzymes that activate a broad range of cell types besides ECs. The prototype member of this group is basic fibroblast

Figure 2. Regulation of angiogenesis by viruses. **A.** Angiogenesis is regulated by a wide variety of pro-angiogenic and anti-angiogenic molecules including growth factors, chemokines, angiogenic enzymes and ECM components. Growth factors, such as VEGF and Ang-2, activate the ECs by binding to specific EC receptors. MMPs degrade the basement membrane (BM) and remodel the ECM, allowing the ECs to migrate and proliferate. This requires the concerted action of soluble factors, ECM components and adhesion molecules. MMPs also increase the biological availability of ECM-sequestered growth factors. During the resolution phase, a new BM is formed, and pericytes are recruited to stabilize the newly formed vessel. Finally, pro-angiogenic factors are down-regulated, and/or anti-angiogenic proteins are produced to keep the endothelium in a quiescent state. During pathological angiogenesis, tumour cells and/or various stromal cell types (i.e., fibroblasts, macrophages and neutrophils) continue to secrete pro-angiogenic and pro-inflammatory factors, resulting in a failure to resolve the angiogenesis cascade. **B.** Viruses may apply direct and indirect mechanisms to deregulate the angiogenesis balance or contribute to tumour angiogenesis. (1) Viruses (e.g. KSHV, HCMV) may directly activate blood vessels as a consequence of endothelial cell tropism. (2) Viruses may produce viral proteins (e.g. HIV-Tat) with direct vascular-promoting activity. (3) Viral infection is often accompanied by acute or chronic inflammation. Inflammatory mediators and recruited immune cells may create an angiogenic microenvironment or act with pro-angiogenic factors to synergistically stimulate angiogenesis. (4) Viruses or viral proteins modulate intracellular signalling pathways that activate the expression of host factors with angiogenic activity

growth factor (FGF2). FGF2 induces EC proliferation, migration and protease production by binding to low-affinity heparan sulphate-containing proteoglycans (HSPG) and high-affinity tyrosine kinase fibroblast growth factor receptors (i.e. FGFR1 and

FGFR2) [9]. The interaction of FGF2 with HSPG promotes FGF2 oligomerization, which is required for the dimerization and subsequent activation of the FGFR. Chemokines are key players in the recruitment of immune cells at sites of infection and/or

inflammation. As such, they may stimulate angiogenesis indirectly. Some chemokines [e.g. interleukin-8 (CXCL8), stromal cell-derived factor-1 (CXCL12)] also possess direct pro-angiogenic activity, whereas others [e.g. platelet factor-4 (CXCL4)] are angiostatic. They exert their action by binding to seven-transmembrane G protein-coupled receptors [10]. Also, pro-inflammatory cytokines (e.g. TNF- α , IL-1, IL-6) have been shown to stimulate angiogenesis. These cytokines mainly act in an indirect manner by up-regulating the expression of direct-acting angiogenic factors. As such, IL-6 enhances the proliferation, migration and tube formation of ECs *in vitro* by up-regulating the expression of FGF2, VEGF, VEGFR-2 and MMPs [11–13]. Enzymes with angiogenic activity include cyclooxygenase 2 (COX-2), angiogenin and thymidine phosphorylase (TP). COX-2 catalyses the production of chemical messengers, such as prostaglandins [14]. Some of these messengers are endowed with pro-inflammatory and/or pro-angiogenic activities. Angiogenin, a member of the ribonuclease superfamily, hydrolyzes cellular tRNAs resulting in decreased protein synthesis. However, the protein also binds to ECs and is transported to the nucleus from where it induces a wide variety of responses including EC proliferation, migration and invasion and protease activation [15]. TP catalyses the phosphorolysis of thymidine to thymine and α -2-deoxy-D-ribose-1-phosphate, which is rapidly converted to 2-deoxy-D-ribose. The latter has been shown to induce EC migration *in vitro* and angiogenesis *in vivo* [16].

Angiogenesis is also regulated by adhesion molecules, such as integrins and cadherins. Integrins mediate the attachment of cells to ECM proteins. In particular, $\alpha_v\beta_3$, which is a receptor for a number of proteins with an arginine-glycine-aspartic acid (RGD) sequence (e.g. fibronectin, vitronectin, laminin) plays a key role during angiogenesis [17]. Vascular endothelial (VE)-cadherin determines the integrity of the endothelial layer [18]. Consequently, degradation of VE-cadherin induces EC apoptosis and vascular permeability.

The activity of these pro-angiogenic factors is counterbalanced by endogenous inhibitors of angiogenesis. These anti-angiogenic molecules assure a tight regulation of the angiogenesis cascade and serve to keep the ECs in a quiescent state when neovascularization is not needed. They include

tissue inhibitors of MMPs (TIMPs), anti-angiogenic chemokines (e.g. CXCL4), thrombospondins (TSP) and various molecules that are derived from the ECM (e.g. angiostatin, endostatin) [4–6,10].

Accumulating data indicate that angiogenesis is not only regulated by hypoxia or cellular factors but also by various viruses, which either express their own pro-angiogenic factors or modulate the activity of cellular proteins (Figure 2). This review focuses on those human viruses for which a (clear) link with angiogenesis has been established (Table 1).

HUMAN TUMOUR-INDUCING VIRUSES

So far, at least six viruses have been associated with human cancer. These include the DNA viruses Epstein-Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), human papilloma virus (HPV), HBV and the RNA viruses, HCV and human T-cell lymphotropic virus type 1 (HTLV-1). These viruses account for 10%–15% of the cancers worldwide [19].

Epstein-Barr virus

The herpesvirus family consists of large double-stranded DNA viruses, which are divided into three subfamilies: α -herpesviruses, β -herpesviruses and γ -herpesviruses. After primary infection, these viruses usually establish life-long latency. In immunocompetent people, latent infection is typically asymptomatic. However, the γ -herpesviruses EBV and KSHV, which preferentially infect B lymphocytes, are associated with malignancies of lymphoid, epithelial, and endothelial origin [19].

Epstein-Barr virus pathology

Epstein-Barr virus was isolated more than 40 years ago from a B-cell lymphoma and was the first virus for which a direct association with human cancer was established. The virus induces multiple malignancies, including Burkitt's lymphoma, Hodgkin's lymphoma, nasopharyngeal carcinoma (NPC) and gastric carcinoma [19,20]. In EBV-induced malignancies, EBV infection is predominantly latent. During latency, only a subset of viral genes, that is, six EBV nuclear antigens (EBNAs) and three latent membrane proteins (LMFs) are expressed, which allows the virus to persist in the host without being recognized by the immune system.

Table 1. Angiogenic activity of human viruses

Virus	EC tropism	Angiogenic activity	Pro-angiogenic host factors* induced or activated by the virus and/or viral proteins				Reference
			Growth factors/receptors	Cytokines/chemokines	Proteolytic enzymes	Other	
Human tumour-inducing viruses							
EBV	No	–	VEGF, FGF2	CXCL8	MMP-1, MMP-9	TP, COX-2	[21,23,25,30–33]
KSHV	Yes	Yes	VEGF, VEGF-C, FGF2, Ang-2	IL-1 α , IL-6, CXCL8, CXCL1	MMP-1, MMP-2, MMP-7, MMP-9	TP, COX-2, angiogenin	[39,41–46,51,56,62]
HPV	No	Yes	VEGF, FGF2, PlGF	IL-6, CXCL8	MT1-MMP, MMP-2	–	[74–76,78,80,83]
HBV	No	Yes	VEGF, Ang-2	–	MT1-MMP, MMP-2, MMP-3, MMP-9	COX-2	[89,95,97–100]
HCV	No	Yes	VEGF, Ang-2	–	MMP-2, MMP-9	COX-2	[105,108,111]
HTLV-1	No	Yes	VEGF, FGF2	–	MMP-2, MMP-9	–	[113–115]
Viruses with a possible role in tumourigenesis							
HIV-1	No	–	VEGF, FGF2	CXCL8	MMP-2, MMP-9	Integrin $\alpha_5\beta_1$, $\alpha_v\beta_3$	[123–125,129]
HCMV	Yes	Yes	VEGF, PlGF, PDGF	IL-6, CXCL1, CCL2, CXCL8	MMP-1, MMP-2, MMP-9, MMP-10, MMP-12	COX-2, angiogenin, β_1 and β_3 integrin	[138–140]
Viruses that are not associated with cancer							
HHV-6	Yes	Inhibition?	–	CCL2, CXCL8	–	–	[149]
HSV-1	No	Yes	VEGF	IL-6	MMP-2, MMP-8, MMP-9	COX-2	[151,153–156]

EGFR, epidermal growth factor receptor.

*Linked to angiogenesis in the referred paper.

Angiogenic activity of Epstein–Barr virus

The EBV LMP1 protein is detected in at least 70% of NPC lesions. Immunohistochemical analysis of NPC tissue samples demonstrated a significant correlation between LMP1 expression and angiogenesis, suggesting that LMP1 increases the production of angiogenic factors [21,22]. In particular, significant correlations have been established between LMP1 and COX-2 [23], MMP-9 [24], CXCL8 [21] and TP [25] levels in NPC. Angiogenesis not only promotes primary tumour growth, it also facilitates the dissemination of tumour cells into the blood and the development of distant metastases. As such, through the induction of angiogenesis, LMP1 may enhance lymph node metastasis of NPC [22]. Moreover, in non-Hodgkin's lymphoma (NHL), the expression of the viral

LMP1 protein was correlated with that of VEGF and with survival rate, suggesting an important role for VEGF in the pathogenesis of EBV-induced NHL [26].

In agreement with the clinical data, LMP1 was found to activate the expression of various pro-angiogenic proteins *in vitro* (Table 1). LMP1 is considered the principal oncoprotein of EBV [20]. It acts as a constitutively active receptor that mimics activated CD40, a member of the TNF receptor family [27]. As such, LMP1 activates several signalling pathways in a ligand-independent manner (Table 2). In particular, LMP1 increases the activity of HIF-1 α in EBV-negative NPC cells, resulting in enhanced expression of the major HIF-1 responsive gene *VEGF* [28,29]. Nuclear factor-kappaB (NF- κ B) is involved in LMP-1-induced expression of FGF2 [30],

Table 2. Viral proteins with angiogenesis-modulating function

Virus	Angiogenic activity involved in pathology	Viral proteins with potential angiogenic activity	Signalling pathways involved	Reference
EBV	Nasopharyngeal carcinoma	LMP1	HIF-1 α , MAPK, NF- κ B, JAK/STAT	[25,28,163]
	Burkitt's lymphoma	EBNA1	MAPK, HIF-1 α , STAT-1	[34,164]
KSHV	Kaposi's sarcoma	LANA	MAPK, HIF-1 α	[50,165]
		vCCL1, 2, 3	ND	[54,55]
		vGPCR	VEGFR-2, HIF-1 α , JAK/STAT, MAPK, NF- κ B, PI3K/Akt	[62–67]
		vIL-6	MAPK	[58]
		vFLIP	MAPK, NF- κ B	[51]
		vIRF3	HIF-1 α	[59]
		K1	PI3K/Akt	[36,60]
HPV	Cervical carcinoma	miRNA	–	[52]
		E5	MAPK, PI3K	[79]
		E6	HIF-1 α	[80]
		E7	HIF-1 α	[80]
HBV	Hepatocellular carcinoma	HBx	HIF-1 α , MAPK	[91,92]
HCV	Hepatocellular carcinoma	Core? NS5A? E2?	–	[110,111]
HTLV-1	Adult T-cell leukaemia	Tax?	–	[115]
HIV-1	AIDS-Kaposi's sarcoma	Tat	VEGFR-2, integrins, MAPK	[121,123,125,127]
HCMV	Transplant vascular sclerosis	US28	NF- κ B	[135,143]
HHV-6	–	Anti-angiogenic U94	–	[148]
HSV-1	Herpetic stromal keratitis	–	–	[151]

CXCL8 [21] and TP [25]. Moreover, LMP1 stimulates the expression and activity of several matrix metalloproteinases in transfected, EBV-negative, NPC cells [31,32]. Transgenic mice that express LMP1 in the epithelial cells display increased levels of MMP-9 with increasing vascularization in the skin, indicating that MMPs are, at least in part, responsible for the angiogenic activity of LMP1 [33].

Like LMP1, the viral EBNA1 protein is expressed in all EBV-associated tumours. EBNA1 activates the transcription factor AP-1 in epithelial cells, resulting in increased expression of AP-1 target genes, including *HIF-1 α* , *VEGF* and *CXCL8*. Consequently, conditioned medium (CM) from EBNA1-expressing cells enhances angiogenesis *in vitro* [34].

Thus, although a direct angiogenic activity of EBV has not yet been reported and EBV does not infect ECs, the virus, and in particular its latent oncoprotein LMP1, induces the expression of various cellular factors that may stimulate angiogenesis in a paracrine way (reviewed in [35]) (Table 1). Moreover, the angiogenic activity of LMP1 has been directly correlated with aggressiveness (i.e. increased metastasis and/or shorter patient survival) of NPC and NHL in clinical samples [22,26].

Kaposi's sarcoma-associated herpesvirus

Kaposi's sarcoma-associated herpesvirus pathology

Kaposi's sarcoma-associated herpesvirus is the etiologic agent of Kaposi's sarcoma (KS) and two rare forms of B-cell lymphoproliferative diseases: primary effusion lymphoma and multicentric Castleman's disease [19,36]. KS is grouped into four epidemiological forms: classic KS, appearing sporadically in elderly men of the Mediterranean area; endemic KS, which exists in subequatorial Africa; iatrogenic KS, occurring in immunosuppressed persons after organ transplantation; and AIDS-associated KS (reviewed in [36]). Whereas classic KS is a mild disease that is limited to the skin, AIDS-associated KS presents as a highly aggressive tumour that often disseminates to the liver, spleen, gastrointestinal tract and lungs.

KS is a strongly vascularized tumour, characterized by proliferating spindle cells (the KS tumour cells, which are considered to originate from ECs), inflammatory cells and abnormal, leaky, slit-like blood vessels. KSHV is present in the majority of the spindle cells of the lesion, but only few cells (1–5%) express KSHV lytic proteins [37]. However,

these lytic cells are essential for KS development, suggesting an interplay between latent and lytic proteins in establishing KS [38].

Angiogenic activity of Kaposi's sarcoma-associated herpesvirus

Infection of ECs with KSHV induces the transcription of several host genes involved in neovascularization [39,40]. As a consequence, KSHV-infected ECs show increased adhesion, invasion, survival and angiogenesis. KSHV-induced angiogenesis is mediated by various cellular factors, including VEGF [41], VEGF-C (a member of the VEGF family that stimulates lymphangiogenesis) [41], angiogenin [42], Ang-2 [43], COX-2 [44] and several chemokines [45] and proteolytic enzymes (Table 1) [46]. KS lesions, as well as ECs that are latently infected with KSHV, contain increased levels of HIF-1 α and HIF-2 α , due to transcriptional activation by KSHV [47]. Thus, increased HIF activity under normoxia may partly account for the up-regulation of angiogenic genes in ECs after KSHV infection. Infection of ECs with KSHV also induces vascular permeability by degradation of the cell–cell adhesion molecule VE-cadherin [48]. Increased vascular permeability may facilitate virus spread and promote inflammation and angiogenesis, thus contributing to KS progression.

Kaposi's sarcoma-associated herpesvirus genes involved in angiogenesis

Kaposi's sarcoma-associated herpesvirus encodes at least 89 open reading frames, many of which are unique among the *Herpesviridae* and are homologues of cellular proteins involved in cell cycle control, apoptosis prevention and immune regulation. Moreover, several KSHV-encoded proteins possess angiogenic and/or oncogenic activity (reviewed in [19,35] and Table 2).

During the latent cycle, only a few KSHV-encoded genes are expressed, including *LANA*, *vCyclin*, *vFlip*, *Kaposin B* and a microRNA cluster [19]. Present data indicate that these proteins confer a growth advantage to the infected cells, rendering them more responsive to the paracrine effects of KSHV lytic proteins [19,49]. An additional role of these latency proteins may be to induce angiogenesis. Latency-associated nuclear antigen (LANA) was found to inhibit the degradation of HIF-1 α and to stimulate angiogenesis *in vitro* [42,50]. Viral FLICE-inhibitory

protein (vFLIP) increases the expression of various pro-angiogenic proteins (e.g. IL-6, TP, COX-2...) in ECs, possibly via activation of NF- κ B [51]. Moreover, miRNAs of KSHV may stimulate angiogenesis by inhibiting the expression of the anti-angiogenic protein TSP-1 [52]. One of the most up-regulated miRNAs in KSHV-infected ECs and in KS lesions is miR-132, which induces EC proliferation and the formation of tube-like structures *in vitro* [53].

Kaposi's sarcoma-associated herpesvirus lytic proteins that contribute to the angiogenic phenotype of KS include, among others, virally encoded chemokines, viral interferon regulatory factors (vIRFs), vIL-6 and the viral G protein-coupled receptor (vGPCR) (Table 2) [35]. The virally encoded chemokines (vCCL1, vCCL2 and vCCL3) display potent angiogenic activity in the chicken chorioallantoic membrane assay [54,55]. Moreover, lentiviral gene delivery of vCCL2 to ECs stimulated angiogenesis in subcutaneously implanted matrigel plugs in mice. vCCL2 was also shown to up-regulate the expression of various pro-angiogenic factors (e.g. VEGF, IL-1 α) [56]. vIL-6 stimulates tumour growth by increasing vascularization and VEGF production [57]. vIL-6 was also shown to up-regulate Ang-2 and VEGF-C in lymphatic endothelial cells (LECs) [58]. Several other lytic proteins, including vIRF3 and K1, stimulate angiogenesis by inducing VEGF and/or MMP expression [41,59,60]. These viral proteins are expressed during lytic infection, indicating that they act in a paracrine manner to stimulate latently infected KS cells, ECs and/or inflammatory cells in the tumour microenvironment.

Viral G protein-coupled receptor, a constitutively active chemokine receptor, is considered the major oncoprotein of KSHV [61]. vGPCR immortalizes ECs by constitutive expression and activation of VEGF receptor-2 (VEGFR-2) [62]. KS biopsies from patients with AIDS as well as animal models of KS indicate a key role for protein kinase B/Akt in vGPCR-induced angiogenesis and development of KS [63,64]. However, expression of vGPCR in EC also leads to the activation of several other intracellular signalling pathways [e.g. HIF-1 α , mitogen-activated protein kinases (MAPKs), signal transducer and activator of transcription (STAT) and NF- κ B] (Table 2), which, in turn, induce the expression of various pro-inflammatory cytokines (e.g. IL-6) and pro-angiogenic chemokines (e.g. CXCL8), growth factors (e.g. VEGF, VEGF-C) and

proteolytic enzymes (e.g. tissue and urokinase type plasminogen activator) [65–68] (Table 1). Thus, vGPCR signalling may affect every step in the angiogenesis cascade, implying a key role for this lytic protein in KS pathogenesis. Interestingly, vGPCR expression could not be demonstrated in classic KS. In contrast, in AIDS-KS tissues, vGPCR was readily detected together with HIV-Tat protein, suggesting that HIV-Tat may induce vGPCR expression, leading to a more aggressive phenotype (see further) [69].

Human papillomavirus

Human papillomaviruses are double-stranded DNA viruses that cause a wide range of hyperproliferative diseases. Furthermore, persistent infection with high-risk HPV types (e.g. HPV16 or HPV18) causes the development of anogenital cancers, which are characterized by deregulated, high-level expression of the HPV early genes E6 and E7 (reviewed in [70]).

Human papillomavirus infection is associated with increased angiogenesis, both in high-risk HPV-associated cervical dysplasia and cancer and in the more benign HPV-associated warts [71–73]. Although HPV mainly infects epithelial cells, the virus may stimulate neovascularization indirectly by increasing the secretion of angiogenic proteins, which can activate ECs in a paracrine manner (Table 1). As such, VEGF expression and secretion were found to be significantly increased in HPV16-positive cells as compared with HPV-negative cells [74]. Other pro-angiogenic molecules, such as FGF2, CXCL8 and placenta growth factor (PIGF), a VEGF family member, were also up-regulated in HPV16-positive cells, while the expression of the anti-angiogenic TSP-1 and TSP-2 was down-regulated [75,76]. Consequently, CM of HPV16-positive cells or E6/E7-expressing keratinocytes stimulated EC proliferation and migration *in vitro* and angiogenesis in matrigel plugs implanted in mice [75,77]. This supports the hypothesis that these proteins not only contribute to uncontrolled growth of keratinocytes but also to neovascularization required for tumour growth.

High VEGF levels were initially associated with the presence of the E6 viral oncoprotein, but subsequent experiments indicated that E5 and E7 proteins are also implicated in VEGF up-regulation [74,78,79]. VEGF expression induced by E6 and E7 was found to be HIF-1 α dependent (Table 2) [80].

Both HIF-1 α activity and TSP levels are regulated by p53, which is inactivated by E6, suggesting that the angiogenic activity of E6 is mediated by p53 inactivation [75,81,82]. HPV infection may further stimulate neovascularization by increasing the activity of proteolytic enzymes; that is, E7 proteins of the high-risk HPV types induce membrane-type (MT)-1 MMP expression, which in turn activates MMP-2 [83].

Besides these indirect effects of HPV on angiogenesis, some data indicate that HPV may also directly affect ECs. *In vitro*, human cervical carcinoma cells expressing HPV16 E7 release the viral protein into the extracellular compartment [84]. Moreover, recombinant E7 has been shown to enter cervical microvascular ECs and to increase the production of the angiogenic cytokines IL-6 and CXCL8 after prolonged incubation [85]. This effect was not observed when macrovascular ECs were incubated with HPV16 E7, suggesting selectivity of the E7 oncoprotein for capillary endothelium. However, more research is needed to unravel the *in vivo* relevance of these findings. Finally, HPV, generally regarded as a strict epitheliotropic pathogen, has been detected at both DNA and protein levels in ECs of blood capillaries adjacent to oral and cervical cancers [86]. While it seems unlikely that HPV can replicate in ECs, the mechanism by which it is taken up and the possible impact this might have on ECs and on angiogenesis are certainly worth further investigation.

Hepatitis B virus and hepatitis C virus

An estimated 500 million people worldwide are chronically infected with HBV or HCV. Chronic HBV and HCV infections and associated liver cirrhosis represent major risk factors for hepatocellular carcinoma (HCC), being implicated in more than 70% of HCC cases (reviewed in [87]). Although the relationship between HCC and persistent HBV or HCV infection has been well documented, an understanding of their exact role in HCC formation is difficult to establish as hepatocarcinogenesis is a multi-factorial process that may require decades [87]. Recent data indicate that virus-induced stimulation of angiogenesis may contribute to the pathogenesis of HCC, which is a highly vascularized tumour (see below and [88]).

Hepatitis B virus

Hepatitis B virus is a small DNA virus of the *Hepadnaviridae* family that does not express a typical

oncogene. However, insertional activation of cellular proto-oncogenes by integration of HBV DNA, long-term immune-mediated effects and the regulatory protein HBx may contribute to HBV-induced hepatocarcinogenesis. In addition, HBx has been shown to possess pro-angiogenic activity. When mice were injected subcutaneously with matrigel containing either hepatoma cells transfected with HBx or with an empty vector, significantly more blood vessels were observed in the matrigel containing HBx transfectants. Moreover, VEGF expression was shown to be increased in HBx-expressing hepatoma cells both under hypoxic and normoxic conditions [89]. VEGF expression was also up-regulated in the liver of HBx transgenic mice [90]. HBx stimulates angiogenesis through transcriptional activation and stabilization of HIF-1 α [91,92], which in turn activates VEGF transcription (Table 2). Up-regulation of VEGF and HIF-1 α by HBV might play an important role in the clinical setting because patient studies have shown that VEGF contributes to angiogenesis in all stages of HCC [93] and that increased HIF-1 α levels in HCC samples are correlated with poor prognosis [94]. HBx protein also stimulates the expression and secretion of Ang-2 in liver tissue [95]. Because VEGF and Ang-2 expression are positively correlated with microvessel density in HBV-induced HCC [96], it seems likely that stimulation of both angiogenic factors by HBx contributes to HBV-associated HCC. Finally, HBx may contribute to HCC angiogenesis by increasing the production of MMPs [97–100] and COX-2 [97].

Thus, although HBV does not infect ECs, angiogenic factors produced by HBV-infected hepatocytes may stimulate liver angiogenesis in a paracrine way (Table 1). Indeed, CM of HBV-infected hepatocytes was shown to induce tube formation of ECs *in vitro* [101].

Hepatitis C virus

Hepatitis C virus is a positive strand RNA virus belonging to the *Flaviviridae*. In contrast to HBV, HCV is unable to integrate into the host genome, which suggests that inflammatory and/or angiogenic events and viral protein expression are more important in HCV-induced hepatocarcinogenesis.

Chronic hepatitis C patients were shown to contain high levels of angiogenesis markers, such as VEGF, Ang-2 and soluble Tie-2 in the serum, which were significantly reduced by antiviral therapy [102]. Moreover, the number of newly formed

blood vessels was directly related to fibrosis stage in liver biopsies of these patients [103]. Interestingly, HCC tissue samples from HCV-infected patients were shown to be even more vascularized than HBV-associated HCC [104].

Experimental studies showed that CM from HCV-infected Huh7 hepatoma cells is more angiogenic than CM of uninfected Huh7 cells in the chicken chorioallantoic membrane assay [105]. Moreover, HCV gene expression stabilizes HIF-1 α under normoxic conditions. This finding could be confirmed in Huh7 cells expressing the HCV subgenomic replicon, indicating that HIF-1 α stabilization is mediated by (a subset of) HCV non-structural genes [105]. Consequently, expression and secretion of VEGF, a HIF-1 α target, by Huh7 cells was markedly increased after HCV infection (Table 1) [105]. HCV-induced reactive oxygen species were shown to stabilize HIF-1 α by activation of MAPK, PI3K, STAT-3 and NF- κ B signal transduction pathways [105,106]. While these authors showed that the non-structural proteins were sufficient for the increase in VEGF expression, also core-expressing Huh7 cells were found to stimulate VEGF expression and secretion [107].

Furthermore, HCV induces the expression of Ang-2 [108]. Also, MMP-2 could be up-regulated by the presence of the HCV core protein [109] or by binding of the HCV envelope protein E2 to the CD81 receptor present on hepatic stellate cells [110]. Finally, COX-2 expression and activity were induced in core-expressing or NS5A-expressing hepatocytes, whereas core, but not NS5A, exerted a stimulatory effect on MMP-9 levels [111]. These findings were supported by clinical studies, which showed that patients with HCV chronic liver disease (seronegative for HBV) have an increased intrahepatic content of COX-2, MMP-2 and MMP-9 [111], which, along with VEGF and Ang-2, could play an important role in HCV-induced angiogenesis in HCC.

Finally, it should be noted that inflammatory mediators as well as immune cells that are recruited to the inflamed liver tissue may play a significant part in the stimulation of angiogenesis by HBV or HCV infection (Figure 2) [87].

Human T-cell lymphotropic virus type 1

Human T-cell lymphotropic virus type 1 is the etiological agent of adult T-cell leukaemia (ATLL), an aggressive malignancy of activated T cells characterized by frequent visceral invasion of tumour

cells (reviewed in [112]). The invasive nature of HTLV-1-associated disease suggests a close interaction between HTLV-1-infected cells and ECs. *In vitro*, HTLV-1-transformed cells induce the production of MMP-2 and MMP-9 by ECs and the down-regulation of TIMPs [113]. Moreover, CD4⁺ T-cell lines constitutively expressing HTLV-1 secrete higher levels of VEGF and FGF2 and are more angiogenic than HTLV-1 negative cells [114,115]. Accordingly, plasma samples from ATLL patients showed very high levels of VEGF and FGF2 [113]. Reducing the HTLV-1 proviral load resulted in decreased VEGF plasma levels in ATLL patients [116]. Furthermore, an increased microvessel density was observed in several organs from ATLL patients as compared with normal organs from matched controls, suggesting that angiogenesis plays an important role in ATLL progression [117].

Few data are available on the underlying molecular mechanisms behind these findings. The viral transcriptional activator protein Tax was proposed to be sufficient for VEGF up-regulation [115], but these findings were later contradicted [118]. Thus, more research is needed to identify the viral factors implicated in angiogenesis stimulation by HTLV-1.

VIRUSES WITH A POSSIBLE ROLE IN TUMOURIGENESIS

Human immunodeficiency virus

Several clinical features of AIDS cannot be solely explained by infection of CD4⁺ cells by HIV-1. In particular, KS is the most common malignancy in HIV-1-infected, untreated individuals. KS is associated with KSHV infection (see above), but HIV-1 may act as a co-factor in KS pathology (reviewed in [36]). Early studies showed that transgenic mice expressing the HIV-1 *tat* gene develop skin lesions resembling KS [119].

Human immunodeficiency virus-1 trans-activating factor

Human immunodeficiency virus-1 Tat is a viral trans-activating factor required for viral replication and expression of all HIV-1 transcripts [120]. Tat is released during acute infection of T cells by HIV. However, the protein may be internalized and activate the expression of various cellular genes in infected and non-infected cells (reviewed in [121]).

The action of extracellular Tat is mediated by different functional regions that interact with a variety of soluble molecules, ECM components and cellular receptors (reviewed in [122]).

Biological activity of different Tat domains. After its release, Tat binds, *via* its basic domain, to HSPG, which are expressed on the surface of ECs and in the ECM [123]. HSPG act as low-affinity receptors for various growth factors [9]. Hence, by competing for the same heparin-binding sites, Tat may mobilize FGF2 from the ECM. Soluble, bioactive FGF2 will subsequently stimulate EC proliferation and angiogenesis [123,124]. Moreover, Tat binds to and activates the VEGF receptor VEGFR-2, which is present on ECs and on KS cells [125]. As such, Tat mimics some of the biological actions elicited by VEGF, including stimulation of EC proliferation and migration *in vitro* and angiogenesis and vascular permeability *in vivo* [125,126].

Tat also activates integrins, a family of heterodimeric cell-surface receptors that mediate cellular adhesion to the ECM [124]. A well-known recognition motif that is used by several integrins to bind their specific ECM ligand is the RGD sequence, which is also present in Tat. Consequently, Tat may mimic the effect of ECM molecules, such as fibronectin and vitronectin, which promote EC adhesion to integrins $\alpha_5\beta_1$ and $\alpha_v\beta_3$, respectively. The interaction of Tat with these integrins promotes the adhesion, growth and migration of ECs *in vitro* and angiogenesis *in vivo* [124,127].

A 20-residue core domain of Tat containing seven cysteine residues was found to possess potent angiogenic activity [128]. This region is also involved in Tat-mediated recruitment of neutrophils, which secrete various angiogenic molecules, including CXCL8, VEGF and MMPs, raising the possibility that neutrophils attracted by Tat contribute to its angiogenic activity [129].

Interaction of Tat with inflammatory cytokines. In contrast to other growth factors, Tat only acts on primary ECs that are activated by pro-inflammatory cytokines, such as TNF- α and IFN- γ . This may be explained by the fact that these cytokines up-regulate the expression of integrins and VEGFR-2 [130–132]. *In vivo*, additional factors are required to confer angiogenic activity to Tat. Injection of Tat alone does not promote angiogenesis nor does it

induce the formation of KS-like lesions in mice [132,133]. However, injection of Tat together with FGF2 or with inflammatory cytokines that induce FGF2 expression results in an angiogenic response [132,134]. This may be explained by the angiogenic activity of FGF2 itself and also by the fact that FGF2 up-regulates the integrin receptors of Tat, resulting in a synergistic effect [132]. Inflammatory cytokines are abundantly present in AIDS patients and may cooperate with Tat to induce angiogenesis in primary KS lesions. This may account for the higher incidence and more aggressive nature of KS in HIV-infected individuals.

Human cytomegalovirus

Human cytomegalovirus pathology

Human cytomegalovirus (HCMV) is a β -herpesvirus that infects about 70% of the world's population. In healthy individuals, HCMV presents as a life-long asymptomatic infection, but in fetuses and immunocompromised individuals the virus may cause severe disease. In particular, HCMV contributes to the pathogenesis of angiogenic diseases, such as restenosis and transplant vascular sclerosis (TVS), which are characterized by chronic inflammation and concentric neointimal smooth muscle cell proliferation, resulting in vessel narrowing and ultimately allograft rejection (reviewed in [135]). Moreover, although HCMV has not been clearly implicated in human cancer, the virus seems to be specifically present in a variety of human malignancies, suggesting a possible association with cancer (reviewed in [136]).

Angiogenic activity of human cytomegalovirus

Human cytomegalovirus may infect different cell types (i.e. ECs, smooth muscle cells, pericytes, macrophages) involved in angiogenesis and stimulate angiogenesis by direct and indirect mechanisms (Figure 2). The viral *UL130* gene has been identified as the major determinant of EC tropism of HCMV [137]. Infection of ECs with HCMV leads to the activation of the epidermal growth factor receptor and integrins β_1 and β_3 , resulting in increased EC proliferation, motility and capillary tube formation [138].

By means of mass spectrometry and cytokine array, it was shown that HCMV induces the expression of various host cell factors implicated in angiogenesis and wound healing, including

cytokines (e.g. IL-6...), chemokines (e.g. CXCL8, CCL2, CXCL1...), growth factors (e.g. VEGF, PlGF, PDGF...) and proteins involved in ECM remodeling (e.g. MMPs, TIMPs) (Table 1). Moreover, virus-free supernatants from HCMV-infected ECs (i.e. secretome) but not from HSV-1-infected cells induced angiogenesis and promoted vessel stabilization and EC survival *in vitro* [139]. IL-6 was found to be the major regulator of HCMV-induced angiogenesis through the up-regulation of the anti-apoptotic protein survivin [140]. Also, in a rat cardiac transplant model, cytomegalovirus-accelerated TVS was associated with the up-regulation of various pro-angiogenic genes [141]. Interestingly, many of these genes were also identified in the HCMV secretome, suggesting that HCMV-induced angiogenesis contributes to TVS disease progression.

Human cytomegalovirus encodes a constitutively activated chemokine receptor US28. US28-overexpressing NIH-3T3 cells show a pro-angiogenic phenotype characterized by increased expression of VEGF [142]. Moreover, recent data demonstrate the up-regulation of COX-2 in HCMV-infected fibroblasts and in US28-overexpressing NIH-3T3 cells [143]. The increase in COX-2 expression was mediated by the activation of NF- κ B and the subsequent production of VEGF (Table 2). US28 activity has also been linked to tumourigenesis, that is, US28 induces a transformed phenotype in and enhances cell cycle progression of NIH-3T3 cells. Moreover, US28-expressing cells promote tumourigenesis when injected into nude mice [142]. The pro-angiogenic and transformed phenotype was not observed in cells expressing a G protein-uncoupled mutant US28, indicating that constitutive activation of G proteins by US28 is required for the observed changes in cell behaviour [142]. Finally, transgenic mice in which US28 is targeted to intestinal epithelial cells display hyperplastic intestinal epithelium and develop adenomas and adenocarcinomas [144].

Together, these data indicate that HCMV contributes to the pathogenesis of vascular diseases by stimulating angiogenesis (reviewed in [135]). HCMV may induce (i) the expression of viral proteins (i.e. US28) with angiogenic activities, (ii) the expression and/or secretion of host angiogenic factors and (iii) inflammation, leading to the recruitment of immune cells, which amplify the angiogenic response by secreting stimulatory factors (Figure 2). Future studies will reveal whether

US28 may be regarded as a viral oncogene, similar to the constitutively activated vGPCR of KSHV.

VIRUSES THAT ARE NOT ASSOCIATED WITH CANCER

Human herpesvirus 6

Human herpesvirus 6 (HHV-6) is a β -herpesvirus, related to HCMV, that causes the benign syndrome exanthema subitum in small children. The virus (in particular, HHV-6B) infects primarily T lymphocytes and subsequently establishes life-long latency. However, HHV-6 frequently reactivates in immunosuppressed patients where it may cause fatal complications, including encephalitis and myocarditis (reviewed in [145]).

Although HHV-6 is a lymphotropic virus, it has been shown to infect and replicate (although at low-level) in vascular and lymphatic ECs [146–149]. HHV-6 replication was demonstrated in adult heart microvascular and aortic endothelium in the absence of cytopathic effects. Moreover, HHV-6 infection of ECs resulted in the up-regulation of CCL2 (monocyte chemoattractant protein-1) and CXCL8, pro-inflammatory chemokines with angiogenic activity (Table 1) [149]. However, recent data by Caruso *et al.* [148] indicate that infection of (lymphatic) ECs with HHV-6 results in the inhibition of angiogenesis, as shown by the inability of the infected cells to migrate and to form capillary-like structures *in vitro*. The anti-angiogenic effects were shown to be associated with the expression of HHV-6 *U94/rep*, a latency-associated gene [148]. Further studies are required to establish the relevance of these data to HHV-6 pathology. However, because lymph nodes are a site of HHV-6 persistence, lymphatic ECs may be more relevant to HHV-6 biology than vascular ECs.

Herpes simplex virus-1

Herpes simplex virus-1 (HSV-1) is a ubiquitous α -herpesvirus that is usually acquired during childhood. Primary infection is often subclinical or asymptomatic, after which latency is established. One of the more distressing complications caused by HSV-1 infection is herpetic stromal keratitis, which is associated with vision impairment and blindness resulting from chronic inflammation and subsequent scarring of the cornea (reviewed in [150]).

Infection with replication-competent HSV-1 induces corneal angiogenesis associated with VEGF expression [151]. Early after infection, VEGF is evident in corneal epithelial cells, whereas in the subsequent clinical phase, inflammatory cells (i.e. neutrophils and macrophages) are the only sites of VEGF production. IL-6 produced by virus-infected cells was found to stimulate non-infected corneal and inflammatory cells in a paracrine manner to secrete VEGF [152]. HSV-1 infection of the cornea was also shown to modulate the expression of the proteases MMP-2 and MMP-9 and their endogenous inhibitors TIMP-1 and TIMP-2 (Table 1) [153,154]. MMPs are up-regulated when keratitis becomes evident, indicating that they participate in the destruction of the cornea after HSV-1 infection, whereas TIMPs are expressed during the healing process [154]. MMP-9 production requires neutrophil invasion into the cornea by replication-competent virus. In addition, HSV-1 infection of human keratocytes resulted in a selective down-regulation of the anti-angiogenic TSP-1 and TSP-2 [155]. A critical role has also been suggested for COX-2 in HSV-1-induced keratitis [156]. The administration of a COX-2 inhibitor after HSV-1 infection resulted in reduced (i) neutrophil infiltration (ii) VEGF levels and (iii) angiogenesis in the cornea, and diminished herpetic stromal keratitis severity.

Thus, HSV-1 infection disrupts the normal equilibrium between angiogenic and anti-angiogenic factors in the (normally avascular) cornea leading to neovascularization. Unlike some other herpesviruses, HSV-1 does not appear to encode pro-angiogenic proteins. Instead, HSV-1 infection causes uninfected, neighbouring cells to express various angiogenesis stimulators [151,156]. The angiogenic response is further increased by infiltrating inflammatory cells, which are a major source of proteolytic enzymes.

CONCLUDING REMARKS

The viruses reviewed here utilize diverse biological pathways to regulate blood vessel formation, including (i) direct activation of ECs as a consequence of EC tropism, (ii) modulation of host gene expression by the activation of various host signalling pathways, (iii) expression of viral proteins with angiogenic activity and/or (iv) induction of inflammation (Figure 2). Despite these differences, several mechanisms to induce neovascularization are shared by viruses belonging to distant families.

Of importance is the finding that all human viruses known to be tumourigenic increase the activity of the transcription factor HIF-1 (the major regulator of oxygen homeostasis) under normoxic conditions (Figure 1). By stabilizing the oxygen-sensitive HIF-1 α subunit, these viruses mimic the hypoxic condition, resulting in increased expression of various angiogenic factors under normoxia. Consequently, these viruses may deregulate the angiogenic balance in normoxic tissue and turn on the angiogenic switch in small, avascular tumours. Vascularization of the tumour will allow further tumour growth and facilitate spreading of tumour cells to distant organs. Thus, these viruses not only induce oncogenesis, but their pro-angiogenic properties may also contribute to a more aggressive tumour phenotype.

A pro-angiogenic mechanism that applies to most, if not all, viruses is induction of inflammation. This may be particularly relevant for viruses that are associated with chronic inflammation, such as HBV and HCV. Viruses typically induce an inflammatory response, which is accompanied by the release of pro-inflammatory proteins and the attraction of immune cells, which secrete various pro-angiogenic factors. A key player in the activation of a pro-inflammatory phenotype is the NF- κ B pathway, which is activated by viruses and is involved in the up-regulation of cellular proteins with angiogenic activity. Thus, inflammation and angiogenesis are tightly linked, and by inducing inflammation, viruses create an angiogenic environment that may favour the development/progression of angiogenic diseases or cancer.

Host factors that contribute to neovascularization and are induced by all viruses (mentioned here) are MMPs. These proteolytic enzymes are thought to facilitate spreading of the virus through tissues. However, remodelling of the ECM is also an essential step of the angiogenic cascade. Thus, a mechanism applied by viruses to promote spreading may also be utilized by ECs to invade and migrate in the extravascular space.

Finally, it should be noted that the knowledge of angiogenesis regulation by viruses is, apart for KSHV and HIV-Tat, still limited. Here, we only discussed those human viruses for which a (clear) link with angiogenesis has been established. However, recent data indicate that many more viruses may create an angiogenic phenotype; for example rhinoviruses were shown to induce

angiogenesis by increasing the expression of VEGF [157], the clinical relevance of which remains to be determined. Adenoviruses and adenoviral vectors stimulate neovascularization, a finding that warrants further investigation, in view of their potential future use in cancer therapy [158–160]. Moreover, Dengue and Hanta viruses, which induce capillary permeability and subsequent microvascular leakage, stimulate the expression of cellular factors with pro-angiogenic activity (e.g. MMPs, Ang-2, CXCL8) [161,162]. Viruses may also inhibit blood vessel formation, as shown recently for HHV-6. Thus, various viruses may disturb the delicate balance between angiogenic and angiostatic proteins that exists in healthy tissues, the outcome of which largely depends on the tissue

and cell type that is infected and/or the viral proteins being expressed.

CONFLICT OF INTEREST

The authors have no competing interest.

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