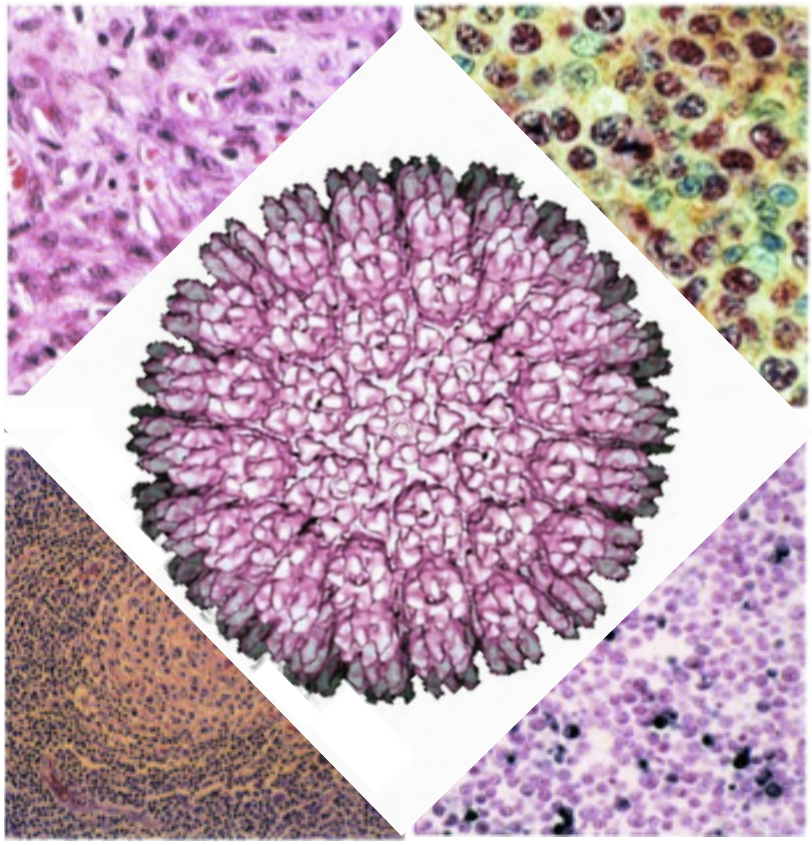
Oncogenic herpesviruses: viral mechanisms and modified immune responses involved in oncogenesis



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1 November 2012

Abstract

The herpesviruses EBV and KSHV are strongly associated with malignancies like Hodgkin's lymphoma, Kaposi's sarcoma and Burkitt's lymphoma. Distinguishing between an associative or causative relation however is very challenging. In this review a minimal set of guidelines to assist with this distinction is extracted from various etiologic guidelines. Using these guidelines the strength of associations between EBV or KSHV and their linked malignancies is shown. Furthermore the KSHV and EBV gene expression profiles and oncogenic capacities of these genes are described in detail. Together this information is used to determine causality for EBV and KSHV in their related malignancies. However, the important role of immune evasion cannot be neglected as KSHV and EBV infection alone does not seem to be sufficient to cause cancer. The immune evasion mechanisms of EBV and KSHV viruses and their associated malignancies are discussed and further research is proposed to obtain more insight into the molecular mechanisms changed by this immune suppression. A better understanding of the role of immune suppression in the cause of malignancies can contribute to develop specific treatments of these EBV- and KSHV-associated tumors and to determine possible cancer prevention strategies.

Box 1	**Commonly used abbreviations**
BL	Burkitt's lymphoma
HL	Hodgkin's lymphoma
NPC	Nasopharyngeal carcinoma
PTLD	Post-transplant lymphoma disease
GC	Gastric cancer
KS	Kaposi's sarcoma
PEL	Primary effusion lymphoma
MCD	Multicentric Castleman's disease
AIDS	Acquired immunodeficiency syndrome
BCR	B cell receptor
CTL	Cytotoxic T cell
ER	Endoplasmic reticulum
FLIP	FLICE-like inhibitory protein
IAP	Inhibitor of apoptosis
IL	Interleukin
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
P53	Protein 53
pRb	Retinoblastoma protein
TAP	Transporter associated with antigen presentation

Contents

1. Introduction

Cancer is the general name for a large group of complex and multi-step diseases that are all caused by unregulated cell growth. Currently cancer forms a big threat for human beings. In 2008, 7.6 million people worldwide died from cancer, which is equal to 13% of all deaths in that year. Cancer is thereby a leading cause of death worldwide.[1] In agreement cancer accounts for nearly 1 of every 4 deaths in the USA and is the second most common cause of death, exceeded only by heart disease.[2]

In the past couple of decades experimental and epidemiologic data indicate that certain viruses have the capability to facilitate or even cause cancer by contributing to different steps in the oncogenic process.[3] The viral presence in its related malignancies ranges from 15 to 100% depending on the virus, the cancer and the geographic location.[4] In total oncogenic viral infections are responsible for approximately 15% of the worldwide cancer incidence. Currently oncogenic viruses are the second leading cause for cancer development in humans, topped only by smoking tobacco, the main cause of cancer development.[5]

As viral infection plays a key role in cancer development, treatment and prevention of viral-induced oncogenicity can benefit the treatment of cancer. Prevention of viral-induced oncogenesis, however, requires understanding of the oncogenic mechanisms that are performed by oncoviruses. From various studies it is known that both single-stranded (ss) RNA as well as double-stranded (ds) DNA viruses have oncogenic potential.[6] Based on the differences in genome, three general oncogenic mechanisms can be distinguished between RNA retroviruses, small DNA viruses, and large DNA viruses.

At the moment only one member of the ss-RNA human retroviruses, HTLV-1, is known to have oncogenic potential. As retroviruses, during their life cycle, insert their genome randomly into the host genome, this creates a potential oncogenic outcome. Viral genome insertion could lead to an upregulation of pro-oncogenetic oncogenes or a downreglation or even knock-out of anti-oncogenetic tumorsuppresor genes.[3]

The small human DNA tumor viruses, including human papillomaviruses (HPV), human polyomaviruses (JVC, BKV, SV40) and hepadnaviruses (HBV and HCV), on the other hand, lack replication factors like DNA polymerase and therefore need to hijack (part of) the cellular replication mechanism. In order to obtain these required replication factors, small DNA tumor viruses therefore activate the cell division cycle. Overstimulation of this cell cycle can contribute to oncogenesis.[7]

Large human DNA viruses, like herpesviruses, on the other hand contain a larger genome and supply their own replication factors, which makes them independent of the host cell cycle status. Their oncogenic character is still extensively researched and varies by herpesvirus specie.[4]

In this review current research into the oncogenic mechanism of the human herpesviruses EBV and KSHV will be reviewed. The viral expression patterns, oncogenic role of viral proteins and the inhibiting role of the immune system on oncogenesis will be extensively described. Finally, some proposed models on the relation between immunodeficiency and the onset of cancer in virally infected patients will be discussed.

2. Determination of an etiologic role for oncoviruses

Already in 1908 Ellerman and Bang reported that viral infection could lead to the development of leukemia.[8] Since that time, many associations between viruses and cancer have been discovered, based on epidemiologic studies and the detection of viral molecules in the tumor, like viral DNA, RNA, proteins or occasionally entire viral particles. With increasing technical equipment and techniques such as the confocal microscope and better immunohistochemistry, the detection of viral molecules in tumors becomes more accurate Nevertheless determination of a causal relationship between viral infection and oncogenesis remains challenging. General guidelines for the determination of a causal relationship between a microbe and a disease were already proposed in 1890 by Koch and are known as the often cited Koch's postulates. These postulates are listed in table 1.[5]

Table 1
Koch's postulates[5]

• The organism must be regularly associated with the disease and its characteristic lesions.
• The organism must be isolated from the diseased host and grow in culture.
• The disease must be reproduced when a pure culture of the organism is introduced into a healthy, susceptible host.
• The same organism must be reisolated from the experimentally infected host.

Viruses were however not known at the time of the development of Koch's postulates and since viruses are, unlike other microbes, incapable of growing in pure virus culture they cannot adhere to Koch's postulates. In addition some viruses, for example HIV, are unable to infect animals, and thereby incapable of fulfilling the reproduction requirement of Koch's postulates. Furthermore many viruses are known that do not cause illness in all infected individuals and the same viral infection can sometimes lead to the onset of various diseases in different individuals.[2,9] While the presence of viral antibodies in the infected host might indicate a co-occurence between the virus and cancer; it is not tight evidence for a causal relation. This also applies to the presence of viral DNA in a human tumor.[3,5]
Butel *et al*. modified tables of Evans and Muller and zur Hausen, containing problems in the determination of the causal role of an oncovirus in human cancer, and constituted 10 barriers that are listed in table 2.[4,6]

Table 2
Barriers to determining an etiologic role of an oncovirus in human cancer[6]

• Although the virus can be universal, cancer is rare
• Usually a long time interval exist between viral infection and tumor causation
• The date of the initial viral infection may be unknown
• Host factor that are significant determinants in susceptibility to cancer vary between individuals
• Different virus strains can have different biological properties, including oncogenicity
• Cofactors may be required for the viral-related oncogenesis
• The same type of cancer can also be caused by chemical or/and physical carcinogens
• Cancer resources may vary in different geographic regions or/and in different age groups
• Available viral detection tests can be uninformative or/and inaccurate
• Lack of a representative animal model

As all the barriers listed in table 2 indicate the difficulty to distinguish a correlation or a causal connection between a virus and its associated human cancer, various researchers have established criteria to make this distinction. To date, no general solid criteria have been accepted and for that reason al three guidelines, proposed by different researchers, are listed in tables 3-5.

Table 3

Zur Hausen criteria[5,10]

• Epidemiological credibility and evidence that a viral infection represents a risk factor for the development of a specific tumor.
• The regular presence and persistence of the viral genome (or parts of it) in tumor biopsies and cell lines derived from the same tumor type.
• The demonstration of growth-promoting activity of specific viral genes or of virus-modified host cell genes in tissue culture systems or in suitable animal systems.
• The demonstration that the malignant phenotype of the associated tumor cells depends on the continuous expression of viral oncogenes or on viral modification of host cell genes.

Table 4

Evans and Mueller guidelines[3]

Epidemiologic guidelines
• The geographic distribution of viral infections corresponds to the tumor distribution and is adjusted for the presence of known co-factors.
• Viral markers are more present in case subjects compared to the matched control subjects.
• Viral markers precede tumor development, meaning that persons with viral markers have a higher incidence of tumors than those without markers.
• The tumor incidence is decreased by viral infection prevention.
Virologic guidelines
• The virus can transform cells *in vitro*
• The viral genome is present in tumor cells and absent in normal cells
• The virus induces the tumor in an experimental animal

Table 5

Hill criteria[3]

• The strength of association How often is the virus associated with the tumor?
• Consistency Has the association been observed repeatedly?
• The specificity of the association Is the virus uniquely associated with the tumor?
• Temporal relationship Does viral infection precede tumorigenesis?
• Biologic gradient Is there a dose response with viral load?
• Biologic plausibility Is it biologically plausible that the virus could cause the tumor?
• Coherence Does the association make sense with what is known about the tumor?
• Experimental evidence Is supporting laboratory data avaiable?

If the three guidelines are compared, they correspond in general. All three appoint epidemiological evidence as an important criterion. Not just the geographical distribution between the virus and cancer should match, but parts of the viral genome, viral markers, should be consistently present in the tumor cells. Both Evans and Mueller (table 4) and Hill (table 5) however, have an additional criterion; these viral markers must also precede tumor development. Zur Hausen (table 3) on the other hand added the criterion that the malignant phenotype should be dependent on the continuous expression of viral oncogenes or on viral modification of host cell genes. In addition the criteria of Evans and Mueller contain the extra guideline that the prevention of viral infection must lead to a decrease in the associated malignancy incidence. In the comparison between the three guidelines the viral capacity to promote cell growth *in vitro* also matches. The criteria of Hill only mention experimental data without further elaboration, while Zur Hausen cites *in vitro* experiments or *in vivo* studies to suffice. Evan and Mueller explicitly call *in vitro* and *in vivo* experiments two independent guidelines. In addition, Hills guidelines are further expanded with biologic plausibility and coherence: could the virus cause the tumor and does the association makes sense? They also name biologic gradient as a criterion: is there a dose-response relationship with viral load? Altogether the minimal criteria that establish causality are:

- consistency of association between a virus and its related malignancy, which can be established solely on epidemiologic evidence;
- presence of the viral genome and gene products in tumor cells;
- direct oncogenic properties of the virus and the viral ability to transform cells in culture and/or produce tumors in experimental animals.

Fulfilling the minimal criteria does not necessarily establish a causal relationship between a malignancy and a virus, however if these criteria are not met, causality can be reasonably excluded. Each criterion from all three sets can be weighed differently in every malignancy and some virally induced malignancies will need additional guidelines to make the distinction. Therefore determining whether a virus has an etiological role in cancer needs to happen on an individual basis, using the current knowledge of the specific virus and its associated malignancies. To enable this, the current knowledge on EBV and KSHV will be summarized in this review and their etiologic role will be discussed in chapter 8.

3. EBV and its associated malignancies

3.1 Characteristics of EBV

EBV, also named human herpesvirus 4 (HHV-4), belongs to the genus lymphocryptovirus that is a member of the gamma subfamily of the herpesviridae. Mature EBV particles have a diameter of 120 - 180 nm and are enveloped by a lipid bilayer. The ds DNA genome is around 184 kb in length and codes for approximately 85 genes.[11] The main target cells for EBV infection are B lymphocytes (B cells).
EBV was originally identified by electron microscopy from biopsy tissue samples of African children that suffered from Burkitt's lymphoma (BL). EBV was thereby the first virus linked to human cancer.[12] Subsequently, seroepidemiologic studies indicated that more than 90% of humans worldwide are infected with EBV. Viral particles are transmitted in the saliva and infection frequently occurs in the first 15 years of life without any symptoms. However if primary infection occurs during adolescent or mature age this can result in infectious mononucleosis (IM), a self-limiting expansion of B cells, characterized by fever, sore throat and fatigue.[13] In 1997 EBV was classified as a carcinogenic agent by the International Agency for Research on Cancer (IARC) for its connection with various malignancies.[14] These malignancies are briefly described below.

3.2 Burkitt's Lymphoma (BL)

An association between EBV and BL was already set in 1964 when viral EBV particles were detected by the electron microscope in BL biopsies.[12] BL is a B lymphocyte malignancy, which supports the link with EBV, as B cells are the main target cells of EBV infection. Nowadays three BL subtypes are identified: endemic, sporadic, and BL associated with acquired immunodeficiency syndrome (AIDS).
Endemic BL is marked by its geographical distribution around the equator and mainly in Africa. Climate factors and the spread of malaria seem important cofactors for this type of BL. From the 5-10 per 10^5 incidences per year the majority occurs in children till 15 years old. Also men are more susceptible than women as the male to female ratio is 3:1. The presence of EBV in endemic BL is 100%, which means that in 100% of tested endemic BL samples either an immune response against EBV or the presence of viral biomolecules was detected. BL tumors are also monoclonal, indicating that the origin of the tumor arises from one single virus infected cell (table 6).[11]
Sporadic BL occurs worldwide but with a 50-100 fold lower incidence compared to endemic BL.[11] EBV prevalence in this subtype range from 80% in areas of intermediate incidence (e.g. North and Northwest Brazil and North Africa) to 20% in the Western world.[13]
In AIDS-associated BL individuals, also the human immunodeficiency virus (HIV) is present. The EBV presence in this BL subtype is 30-40%. Interestingly in these cases all tumor cells contain EBV genomes, indicating that the EBV virus must have been present in the tumor progenitor cells before those expanded to a malignancy.[11]
All forms of BL are characterized by translocation of the cellular Myc (c-Myc) oncogene into one of the immunoglobulin (Ig) loci due to translocations of this gene.[15] Three distinguished translocations are known: t(8;14)(q24;q32), t(2;8)(p12;q24) and t(8;22)(q24;q11) of which t(8;14)(q24;q32) is the most common form (80%). BL cells lack T cell co-stimulatory and cell adhesion receptors CD45 (ICAM1), CD58 and CD80 (B7). They have downregulated MHC I molecules and low-level expression of TAP1 and TAP2. All of these features indicate immune escape and will be described in detail in chapter 7.[11]

3.3 Hodgkin's lymphoma (HL)

Hodgkin's lymphoma (HL) is a B cell lymphoma with a general incidence of 2–3 per 10^5 cases per year worldwide.[13] Individuals with a history of IM have a 3 times higher risk to develop HL. HL can be subdivides in 4 subtypes: nodular sclerosing (ns), mixed-cellularity (mc), lymphocyte depleted (ld), and lymphocyte predominance (lp), each with a different presence of EBV as shown in table 6.[11]
Interestingly the malignant Hodgkin/Reed–Sternberg (HRS) cells, specific for HL, comprise less than 1% of the total cell population of the tumor. These cells appear as atypical immunoblasts or multinucleated giant cells with prominent nucleoli and marginated heterochromatin and they originate from germinal center B cells. The majority of the tumor consists of non-malignant immunological cells like lymphoid stroma, plasma cells, eosinophils, and granulocytes.[4] These immune cells are recruited by various cytokines (TGFβ, IL-10 and IL-13) that are secreted by the HRS cells.[11]

3.4 Nasopharyngeal carcinoma (NPC)

Although epithelial cells show resistance to EBV infection, EBV can infect nasopharyngeal epithelial cells with low efficiency. Transformation of these cells by EBV can result in nasopharyngeal carcinoma (NPC). NPC has a very low incidence of 0.5 per 10^5 persons a year in Europe and North America, whereas the incidence in China and South-east Asia is around 25 per 10^5 a year. This difference is described to genetic and environmental differences like the high consumption of salted fish in Asia. Also males are more susceptible to develop NPC compared to women as the male to female incidence ratio is 2-3:1.[16]
There is a distinction between keratinizing undifferentiated carcinoma and nonkeratinizing undifferentiated carcinoma of which especially the latter has a high EBV presence (table 6).[17]
Both subtypes of NPC are characterized by the presence of undifferentiated carcinoma cells and major lymphocytic infiltrate. This interaction between carcinoma cells and lymphocytes seems to be crucial for the continuous replication of the malignant cells.[18]

3.5 Post-transplant lymphoma disease (PTLD)

In immunocompromised patients EBV may cause post-transplant lymphoma disease (PTLD). The majority of patients are immunosuppression after organ transplantation, but also HIV-infection or an innate immune dysfunction can be the cause of immune deficiency in these persons. The presence of EBV molecules in tumor tissue exceeds 80% for all subtypes as shown in table 6.
The occurrence of PTLD indicates that the balance between EBV and the host immune system plays an important role in the prevention of malignancies.[13] A disturbed immune system increases the risk of oncogenesis and restoring the balance, by for example reducing immunosuppression or infusing specific targeted autologous T cells, can cure PTLD.[19]

3.6 Other EBV related malignancies

Primary effusion lymphoma (PEL) is mainly considered as a KSHV related malignancy, as the KSHV presence in this lymphoma is 100% (table 7). However, 90% of PELs are co-infected with EBV, making the role of EBV significant.[20]
Two subtypes of T cell lymphomas are distinguished: virus-associated hemophagocytic syndrome (VAHS) T cell lymphomas and the nasal NK/T cell lymphomas. As T cells express

low levels of the EBV receptor, it is possible for EBV to infect these cells, although the efficiency of this process is very low. Both subtypes are monoclonal and the presence of EBV is detected in 100% of the tumor samples (table 6). VAHS T cell lymphomas are rare malignancies associated with hemophagocytosis, whereas nasal NK/T cell lymphomas are aggressive, destructive tumors that like NPC have a high prevalence in populations in Southeast Asia and Central/South America. Little is known yet about the contribution of EBV to the oncogenesis of these malignancies and currently there are no *in vitro* models for these EBV-infected T cell malignancies available.[21]
Besides nasopharyngeal epithelial cells EBV is capable to infect gastric epithelial cells with low efficiency *in vitro*. Subsequently EBV can transform these cells leading to gastric carcinoma (GC). Worldwide, EBV is detected in 10% of GC cases by PCR and *in situ* hybridization. In these EBV positive cases however 100% of the cancer cells are EBV positive and the tumors are monoclonal. As GC is worldwide one of the most common carcinomas, the EBV-infected carcinoma cases are estimated at more than 50,000 cases/year.[22]

Altogether EBV is linked to a broad range of malignancies. These consist mainly of lymphomas, as B cells are the main targets cells for EBV infection, but also NK and T lymphomas and gastric epithelial carcinomas are linked with EBV infection. The association between EBV and these malignancies are summarized in table 6.

Table 6
The spectrum of EBV-associated malignancies[23]

Tumor	Subtype	Typical aclinical period	EBV presence (%)[a]
BL	Endemic	3-8 years post EBV	100
	Sporadic		15-85
	AIDS-associated	3-8 years post HIV	30-40
HL	Mc, ld	> 10 years post EBV	60-80
	Ns		20-40
NPC	Non-keratinizing	> 30 years post EBV	100
	Keratinizing		30-100
PTLD	Immunodeficient	< 3 months post EBV	100
	Post-transplant	< 1 year post transplantation	> 90
	AIDS-associated	> 8 years post HIV	> 80
GC	UCNT	> 30 years post EBV	100
	Adenocarcinoma		5-15
T cell lymphoma	VAHS-associated	1-2 years post EBV	100
	Nasal NK and T cell	> 30 years post EBV	
PEL		?	90

Abbreviation: UCNT, undifferentiated carcinomas of nasopharyngeal type
[a] Fraction of malignant samples in which an immune response against EBV or the presence of viral biomolecules has been detected.

4. KSHV and its associated malignancies

4.1 Characteristics of KSHV

KSHV, also called human herpesvirus 8 (HHV-8), belongs to the genus Rhadinovirus that is a member of the gamma subfamily of the herpesviridae. Mature KSHV particles have a diameter of 120 - 180 nm and are enveloped by a lipid bilayer.[24] The ds DNA genome is around 165-170 kb in length and codes for approximately 86 genes, of which one quarter are involved in immunomodulation. The main target cells for KSHV infection are B cells. Remarkably, the KSHV genome contains a high number of genes that code for hijacked proteins of the host cell, like viral IL-6, Bcl-2, cyclin, G protein-coupled receptor (GPCR) and flice inhibitory protein (FLIP).[25] DNA fragments of KSHV were isolated for the first time in 1994 from a Kaposi's sarcoma (KS) tumor of an AIDS patient by Chang and Moore.[26] For that reason KSHV is the most recently discovered human herpesvirus and is one of two human herpesviruses, along with EBV, which has clearly been associated with cancer. Shortly after its discovery, KSHV was also linked to the lymphoproliferative disorders primary effusion lymphomas (PEL)[27] and multicentric Castleman disease (MCD).[28]

4.2 Kaposi's sarcoma (KS)

KS is not a true sarcoma but a highly vascular malignancy that arises from endothelial lymphatic cells. KS forms vascular channels that are filled with leukocytes thereby resulting in the characteristic red, brown or purple lesions.[13] The classical subtype of KS was already described in 1872 by the Hungarian dermatologist Moritz Kaposi.[29] Since that time three additional subtypes have been identified: endemic or African, iatrogenic or immunosuppression and AIDS-associated or epidemic KS. Especially during the AIDS-epidemic in the 1980's the occurrence of the latter grew extraordinary mainly in homosexual and bisexual HIV-positive individuals. With the introduction of the highly active antiretroviral therapy (HAART) treatment for AIDS patients, the number of individuals suffering from KS dropped dramatically, indicating the important role of HIV in this KS subtype.[30] In 1994 AIDS-associated KS was linked to KSHV by Chang and Moore, indicating that not HIV but KSHV is the potential cause of KS malignancies.[31] KSHV proteins are uniformly detected in the spindle-shaped tumor cells of all four subtypes of KS (table 7).[13] However, using southern blotting detection, the low amount of one KSHV genome copy per KS cell was determined.[8]
The incidence of classic KS is very low, but up to 10-fold higher in elderly Mediterranean men, mainly from Italy, Greece, Turkey, and Israel. The male to female ratio is 15:1.[32]
African KS is an endemic disease commonly seen in Eastern and Central Africa and affecting mainly two age groups: young men with an average age of 35 years and children with an average age of 3 years. This latter indicates an early primary KSHV infection and indeed a seroprevalance study in 1998 in Uganda showed immune responses to at least one KSHV antigen in 37% of children under the age of 5 years. This seroprevalence is increased to 58% for children between 5-9 years.[33] However due to the high prevalence of HIV in Africa independent studies into the etiologic role of KSHV in endemic KS is almost impossible nowadays.
Besides HAART, the reduction of immunosuppressive treatment in posttransplant patients further reduces the development of KS thereby indicating the potential important role of the immune system in KS oncogenesis. Furthermore iatrogenic KS shows ethnogeographic associations, as about 0.4% of transplant patients in the United States and Western Europe develop iatrogenic KS versus 4.0 to 5.3% of renal transplant patients in Saudi Arabia.[32]

4.3 Primary effusion lymphoma (PEL)

PEL was originality named body cavity-based lymphoma (BCBL) and is a malignancy of the B cell characterized by malignant effusion of B cells in the pleural or abdominal cavity. Detection with immunohistochemistry and molecular techniques indicates that KSHV is almost invariable present in PEL cells (table 7) and the number of KSHV genomes per PEL cell, detected by southern blotting, is in the high range of 40 - 80 copies per cell.[30] In addition the majority of PELs are co-infected with EBV (table 6).[20] PELs are however, unlike BL, not associated with c-Myc translocations.[30]

PELs are rare tumors, even in populations with high KSHV seroprevalence, accounting for about 3% of AIDS-related lymphomas and 0.4% of all AIDS-unrelated diffuse large-cell non-Hodgkin lymphomas.[34] PELs are more commonly associated with immune deficiency, in particular in HIV-positive males, but also occur in HIV-negative men and women.[8]

4.4 Multicentric Castleman's disease (MCD)

MCD is formally not a malignancy but a rare lymphoproliferation capable of evolving into a lymphoma.[13] In contrast with localized Castleman's disease, MCD is often associated with multi-organ involvement, polylymphadenopathy and elevated interleukin (IL)-6 levels.[35]

MCD was already described in 1954 by Benjamin Castleman[36] and is nowadays divided into two main subgroups: the plasma cell variant and hyaline vascular variant. The hyalinized vascular variant is characterized by numerous small to medium-sized germinal follicles in the lymph nodes, with hyalinized vessels and a concentrically arranged mantle zone. Plasma cells, on the other hand, are identified by their clock-face nucleus and pale perinuclear cytoplasmic crescent. A mixed form of MCD, with both hyaline vascular and plasma cells, also exists.[35] The hyalinized vascular subtype rarely occurs for MCD and KSHV presence in this subtype is almost never detected (table 7). The plasma cell variant, however, occurs more often and mainly in HIV-infected individuals. The presence of KSHV in MCD is variable and around 100% in AIDS patients compared to around 50% in HIV-uninfected individuals (table 7).[11] Additionally a link between KS and MCD has also been established as 75% of HIV-positive MCD patients and 13% of HIV-negative MCD patients have or will develop KS during the course of their disease.[37]

Table 7

The spectrum of KSHV-associated malignancies.[38]

Tumor	Subtype	KSHV presence (%)[a]
KS	Classical African/endemic Iatrogenic/immunosuppression AIDS-associated/epidemic	100
PEL		100[b]
MCD	Plasma cell variant Hyaline vascular variant	50-100[c] Usually negative

[a] Fraction of malignant samples in which an immune response against EBV or the presence of viral biomolecules has been detected.

[b] 90% of PEL are also co-infected with EBV[20]

[c] 50% in HIV-negative, up to 100% in HIV-positive MCD patients

5 EBV and KSHV expression

5.1 Latent and lytic phase

Herpesviruses all have the capacity to infect cells lytically or latently. In lytically infected cells all viral proteins are expressed and viral replication results in the formation of new viral particles. Finally, after a few hours, the virus will destroy the infected host cell, resulting in the release of newly formed viral particles. The more silent latently infected cells are characterized by an episome, a circularized, extra-chromosomal viral genome. In the latently infected cells only a restricted set of genes are expressed, resulting in persistence of the viral genome and avoidance of immune surveillance recognition. Latently infected cells are not destroyed and no new viral particles are released from these cells.[39] Because of their latent capacity and corresponding immune surveillance escape, herpesviruses persist lifelong in their host. Therefore not surprising, the vast majority of herpesvirus infected human B lymphocytes, the main target cells of both EBV and KSHV, are latently infected.[40]

5.2 EBV gene expression in associated malignancies

In latent EBV transcription six EBV nuclear antigens EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, and EBNA-LP and three latent membrane proteins LMP-1, LMP-2A, and LMP-2B are expressed.[41] In addition two types of EBV-encoded non-translated RNAs are transcribed: EBERs (EBV-encoded RNAs) and BARTs (BamH1-A rightward transcripts).[11] In the EBV genome also two latent miRNA clusters are also identified: miR-BHRF1-1 to miR-BHRF1-3 and miR-BART1 to miR-BART20, together containing 23 distinct miRNAs.[42]
EBV persists lifelong in its latent reservoir: the memory B cell, the only B cell that circulates long enough through the body to fulfill this function. EBV infection in memory B cells is recorded, but the EBV expression pattern in these cells is more restricted compared to infected B cells that are detected in the blood and lymphoid tissues of infectious mononucleosis patients. Based on this observation and the identification of variations in EBV gene expression in EBV-positive tumors and immortalized B cell lines in additional studies,

Table 8
Gene expression profiles for EBV-associated malignancies[40,42,43]

	BL	HL	NPC	PTLD[a]	PTLD[a]	PEL	NK/T L
EBERS	+	+	+	+	+	+	+
EBNA-1	+	+	+	+	+	+	+
ENBA-2	-	-	-	+	-	-	-
EBNA-3 A-C	-	-	-	+	-	-	-
EBNA-LP	-	-	-	+	-	-	-
LMP-1	-	+	+[b]	+	+	-	+
LMP-2 A	-	+	+	+	+	-	+
LMP-2 B	-	+	+	+	+	-	-
miR-BHRF1 1-3	-	-	-	+	-	-	?
miR-BART 1-20	-	+	+	+	+	-	?
Latency program	I	II	II	III	II	I	I/II

blue text, latent gene
[a] PTLD can express both latency II and III.[44]
[b] LMP-1 is detected in only 40-60% of NPC tumors.[45,46]
Abbreviations: ?, unknow; NK/T L, NK and T cell lymphoma

three different latent phases were proposed for EBV-infected cells: latency I, II and III.[41] Each latency expresses its own set of EBV genes as indicated in table 8. In this table the malignancies associated with this EBV expression pattern are shown as well.[40]
Based on the latency and type of B cell, Babcock and co-workers proposed a model in which EBV genome regulation is based on the differentiation state of the B cell, resulting in unique expression patterns (figure 1).[47] This model is well accepted by other researches.[18,40,48]

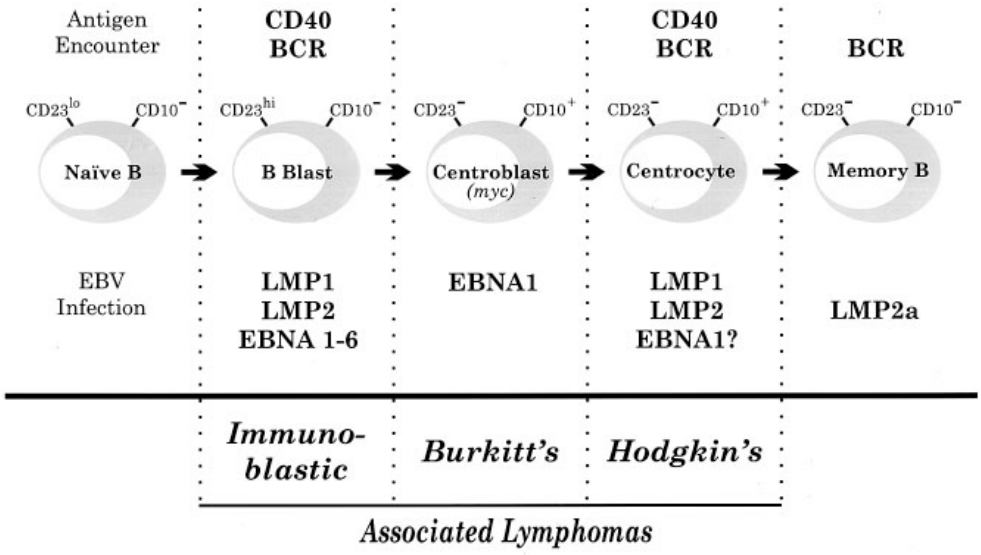

Figure 1: Diagrammatic representation of the B cell differentiation stages between a naïve B cell and its corresponding plasma and memory cell. EBV-associated malignancies and the proposed differentiation stage of their B cell origin as well as their corresponding latent gene expression are also indicated.[47]
(CD10 and CD23, specific B cell differentiation markers)

5.3 KSHV gene expression in associated malignancies

In de novo infection and during initial steps of oncogenesis gene expression patterns of KSHV are unknown.[8] Schulz and Chang, however, combined results from *in situ* hybridization and immunohistochemistry experiments on biopsy samples of KS, MCD and PEL with KSHV gene expression experiments with Northern blot, real time PCR or DNA array on PEL cell lines and biopsy samples to subdivide all approximately 88 currently known genes of KSHV in latent and lytic and early and late expression genes.[23,49] KSHV genes that are expressed during latency are LANA-1, LANA-2, vCyclin, vFLIP and Kaposin B (figure 2).[16] In addition KSHV also contains a miRNA cluster, consisting of 17 distinct miRNAs, which are partially controlled by a latent promoter.[17] In contrast with EBV, no latency subdivisions are known for KSHV. Only LANA-2 is exclusively expressed in PEL and MCD samples, indicating that this gene is B cell specific.
Furthermore KSHV-associated malignancies are characterized by a small percentage (1-5%) of tumor cells that spontaneously switch into the lytic replication cycle. Both KSHV positive cell lines as well as KS, PEL, and MCD biopsies show low levels of lytic expression.[23] KSHV expression patterns per KSHV-associated malignancy are indicated in table 9.[30]

Table 9

In situ detection of KSHV gene products in KS, PEL, and MCD.[30]

	KS	PEL	MCD
LANA-1	RNA and protein	RNA and protein	RNA and protein
LANA-2 (vIRF3)	ND	RNA and protein	RNA and protein[a]
vFLIP	RNA	RNA	RNA
vCyclin	RNA	RNA	RNA
Kaposin B	RNA	RNA and protein	?
vIL-6	RNA and protein[a,b]	RNA and protein[a]	RNA and protein[a]
K1	RNA and protein[a]	RNA and protein[a]	RNA and protein
vGPCR	RNA	RNA	RNA
Rta (ORF50)	RNA	RNA	?
PF-8 (ORF59)	RNA and protein[a]	RNA and protein[a]	RNA and protein[a]
vIRF-1	RNA	RNA	RNA

blue text, latent gene; red text, lytic gene; ND, not detected; ?, unknown;

[a] Protein levels of these viral genes are only detected in a small percentage of tumor cells

[b] vIL-6 expression is highly variable in KS

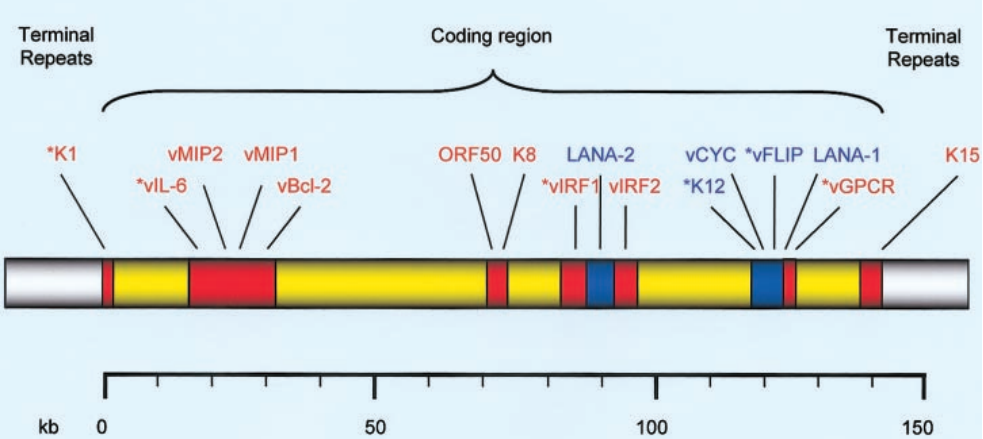

Figure 2: Schematic overview of the KSHV genome. Latent genes are indicated in blue text and lytic genes in red text.[16]

Abbreviations: K12, Kaposin B; vCYC, vCyclin

6. The hallmarks of cancer

Cancer forms a group of complex diseases caused by the transformation of healthy cells into continuously and uncontrolled dividing cancer cells by a complex multi-step process.[50] The two major players in this process are oncogenes, coding for cancer promoting proteins, and tumor suppressor genes, coding for cancer reducing proteins.[51,52] Although there are only two major players, the enormous amount of genes belonging to each group and the large number of possible mechanisms that can influence these genes indicate that the total multi-step process is very complex and not fully understood yet.[53]

The known threshold of oncogenesis are genetic and epigenetic alterations in at least five to eight oncogenes and tumor suppressor genes that are involved in proliferation, invasion and potential metastatic pathways.[49,54] The total multi-step cancer process is also defined as a Darwinian natural selection process, meaning that genetic and epigenetic alterations occur continuously, from which those with proliferative and immune-evading advantages are selected and form the basis of the beginning tumor.[55,56]

Although the complexity of the total cancer process is very high, Hanahan and Weinberg, two founders of current cancer research, have published two influential reviews in which they classify the different aspects of the cancer process in characteristics which they named hallmarks.[57] The intracellular circuit, containing all the pathways that are currently known to be involved in oncogenesis, is presented as a mimic of an electronic integrated circuit in figure 3.[57]

In order to persist in the host lifelong, DNA oncoviruses have developed diverse strategies involved in *inter alia* cell proliferation and cell death inhibition. As these same strategies are also beneficial for cancer development and maintenance, some of the viral strategies match the oncogenesis hallmark requirements. In the following sections interference of EBV and KSHV with carcinogenic processes will be discussed per hallmark.

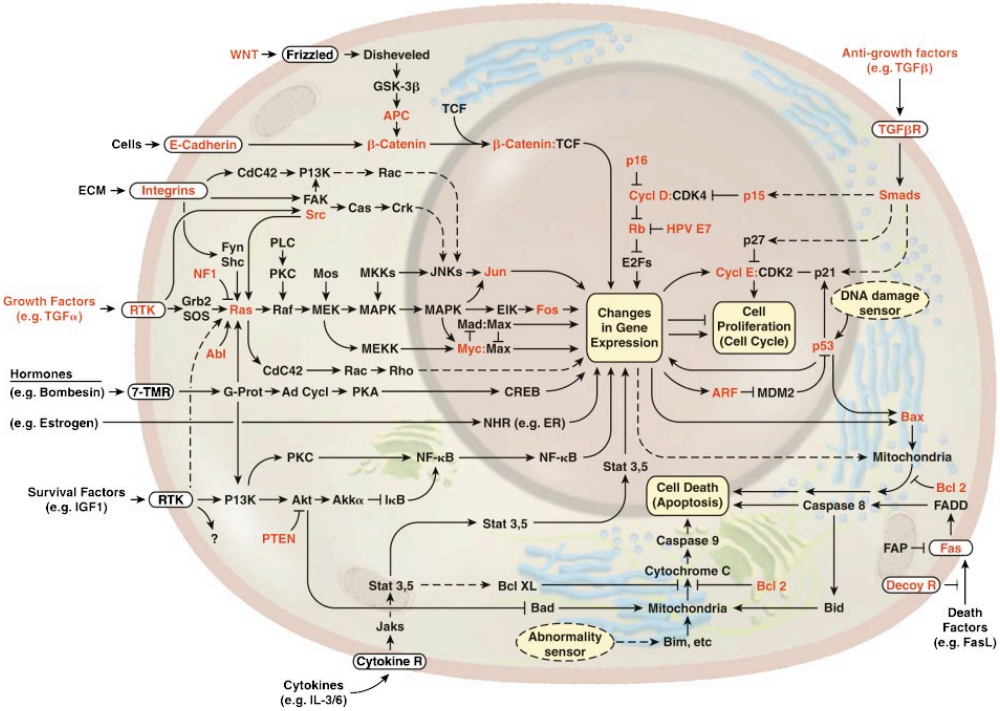

Figure 3: All the proteins, molecules and intracellular pathways that up till now are known to play a role in oncogenesis are indicated in this simplified integrated circuit of the cell. Genes that are known to be functionally altered in cancer cells are highlighted in red.[57]

6.1 Self-Sufficiency in Growth Signals

All human cells require extracellular growth stimuli to progress from an inactive into an active proliferative state. These stimuli can consist of soluble growth factors, cell-to-cell adhesion or interaction components, and extracellular matrix molecules. Cancer cells by contrast are less or even independent of these extracellular growth stimuli. The capacity of cancer cells to continuously proliferate independently of these stimuli indicates their ability to produce their own growth stimuli or adjust the intracellulair circuits targeted by these stimuli. Three main molecular strategies to achieve this self-sufficient state are alterations of extracellular growth-stimulatory factors, of transcellular growth-stimulatory signaling, or of the intracellular pathways responding to these stimuli.[57]

6.1.1 Alterations of extracellular growth-stimulatory factors

Cancer cells can self-synthesize their own growth-stimulatory factors resulting in autocrine signaling. In addition cancer cells may stimulate surrounding cells to produce various growth factors that stimulate the cancer cells, resulting in heterotypic growth-activating signaling.[53]

KSHV stimulates growth promoting IL-6 signaling

KSHV encodes *inter alia* for the growth promoting lytic vIL-6, a 24.8% amino acid identical homologue of the human IL-6 (hIL-6).[58] hIL-6 is known to be an important autocrine/paracrine growth factor, in particular for B cells.[59,60] As vIL-6 is capable of activating the same hIL-6 induced signaling pathways, vIL-6 may function as a soluble growth factor.[61,62] In addition vIL-6 is also capable of inducing the expression of hIL-6, which results in increased hIL-6 levels.[63] Another KSHV encoded latent protein, Kaposin B, also increases IL-6 levels by stabilizing cytokine expression. Kaposin B inhibits degradation of AU-rich cytokine messenger RNA (mRNA) by binding and activation of MK2 kinase.[64] Altogether KSHV infection stimulates growth promoting IL-6 signaling.

6.1.2 Alterations of transcellular growth-stimulatory signaling

Cell surface growth receptors, like epidermal growth factor receptor (EGFR) and tumor necrosis factors alpha receptor (TNFαR), transduce the growth-stimulatory signal to the intracellular pathways. In many cancer cells these receptors are overexpressed or mutated resulting in a hyperresponsive state of the cell to growth stimuli levels that normally would not trigger proliferation.[57]

EBV LMP-1 activates JAK/STAT, JNK and NF-κB pathways

B cells can present antigens to T helper cells, resulting in activation of these T helper cells. The latter can bind via its cell surface expressed CD40 ligand (CD40L) to the tumor-necrosis-factor receptor (TNFR) family member CD40 receptor expressed on the cell surface of the B cells. This binding will cause B cell differentiation in antibody-secreting plasma cells via various pathways as shown in figure 4.[65]
The multiple membrane-spanning LMP-1, a latent protein encoded by EBV, lacks a significant extracellular domain and acts as a constitutively active ligand-independent CD40 homologue receptor.[66] Both the CD40 and LMP-1 intracellular signal cascades are shown in figure 4.

Final outcomes of both pathways are B cell survival and/or cell growth.[67,68] As T helper cells normally bind CD40, LMP-1 is capable of replacing the T cell-derived activation signal and can therefore induce unlimited B cell proliferation.[69]
Interestingly, LMP-1 and CD40 do not interact with the exact same sets of molecules, indicating that their signaling pathways might differ in some aspects (Figure 4).[67] Also worth noting is that shortly after the TRAF recruitment to the cytoplasmic tail of CD40, TRAF2 and 3 undergo degradation, while no degradation is detected for LMP-1-mediated recruitment.[70] Both differences between LMP-1 and CD40 signaling may contribute to the enhanced capacity of LMP-1 to activate B cells and promote B cell transformation.

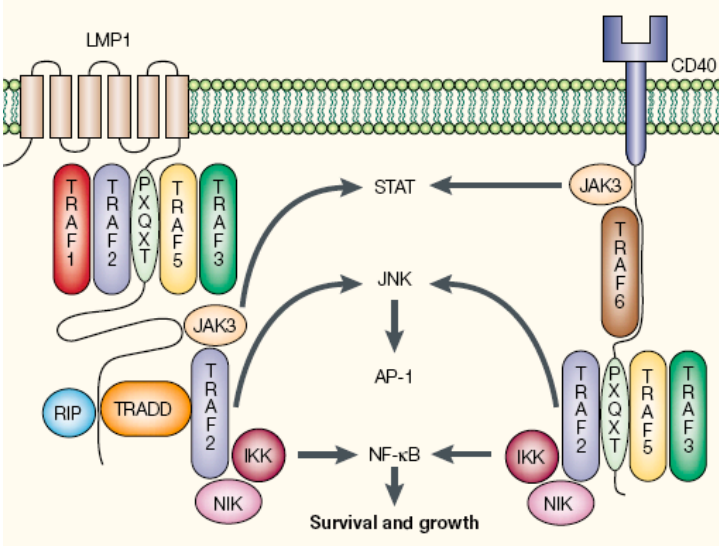

Figure 4: Schematic overview of the signaling relationship between LMP-1 and CD40. Mainly the carboxy-terminal domains of both LMP-1 and CD40 are important for signaling. Both domains interact with distinct TNFR-associated factors (TRAFs) and other binding molecules in the case of LMP-1.[71,72] However both domains contain corresponding TRAF-binding domains and activate the same downstream signaling pathways[73,74] and transcription factors,[75,76] resulting in the identical final outcome of B cell proliferation and/or B cell survival.[67]
Abbreviations: NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; AP-1, activator protein 1; JAK3, Janus-activated kinase 3; STAT, signal transducers and activators of transcription; TRADD, TNFR-associated death domain protein; IKK, IκB kinase.

EBV LMP-1 upregulates EGFR

In addition to the direct growth proliferation function of LMP-1 this EBV encoded protein is also associated with EGFR induction.[77] As LMP-1 is a homologue to CD40, an *in vitro* study in which the cytoplasmic tails of the two proteins were swapped showed that both LMP-1-CD40 chimeric proteins are capable of inducing the same signal transduction including upregulation of EGFR.[78] LMP-1 contains two TRAF binding regions in its cytoplasmic tail termed C-terminal-activating region (CTAR) 1 and 2. *In vitro* studies have demonstrated that the CTAR1 region is responsible for induction of EGFR in epithelial cells.[76] This upregulation is mediated through the TARF signaling pathway.[79] Based on *in vitro* studies with a human cervical carcinoma EBV negative cell line Kung and Raab-Traub composed a molecular mechanism model for LMP-1 induced EGFR upregulation: STAT3 is activated by LMP-1 through the induction of tyrosine and serine phosphorylation by JAK3 as indicated in figure 4. This will lead to increased Bcl-3 expression through interaction with multiple STAT-binding sites in both the promoter and introns of Bcl-3. Together with p50, another protein upregulated by CTAR1 and 2, Bcl-3 forms a transcriptional active complex that transactivates EGFR.[80,81] The important role of activated STAT3 in oncogenesis is supported by *in vitro* and *in vivo* studies with a STAT3 inhibitor that results in a decrease of NPC and invasion.[82,83] The connection with EGFR however is not investigated in these studies. Another *in vitro* study shows that LMP-1 can regulate the nuclear accumulation of EGFR in a dose-dependent manner linking LMP-1 and EGFR.[84] However as STAT3 is not researched in this study it is still waiting for research supporting the model proposed by Kung and Raab-Traub.

KSHV vGPCR activates PI3K pathway

The KSHV encoded lytic G-protein-coupled receptor (vGPCR) can *inter alia* stimulate cellular proliferation and is therefore a potential growth factor receptor. vGPCR is a bona fide signaling receptor with constitutive, agonist-independent activity in the phosphatidylinositol 3-kinase (PI3K) pathway.[85] The complete PI3K/Akt cascade is indicated in figure 5.[86] Final outcomes of this cascade are the stimulation of cell growth, evading of growth suppression and inhibition of apoptosis. Via activation of the PI3K/Akt pathway, vGPCR is involved in all these oncogenic events.[87]

In vivo studies have supported the role of the mTOR pathway (pathway 2, figure 5) in KSHV oncogenesis. Transduction of KS transgenic mice with the vGPCR gene leads to vascular tumor development in which high levels of phosphorylated Akt as well as phosphorylated S6 ribosomal protein, a substrate of S6K1, were detected. In addition immunohistochemical staining of biopsies of cutaneous KS lesions showed high levels of phosphorylated Akt and S6 ribosomal protein in all eight samples tested.[88] Presumable, the mTOR pathway plays an important role in human Kaposi's sarcomagenesis.

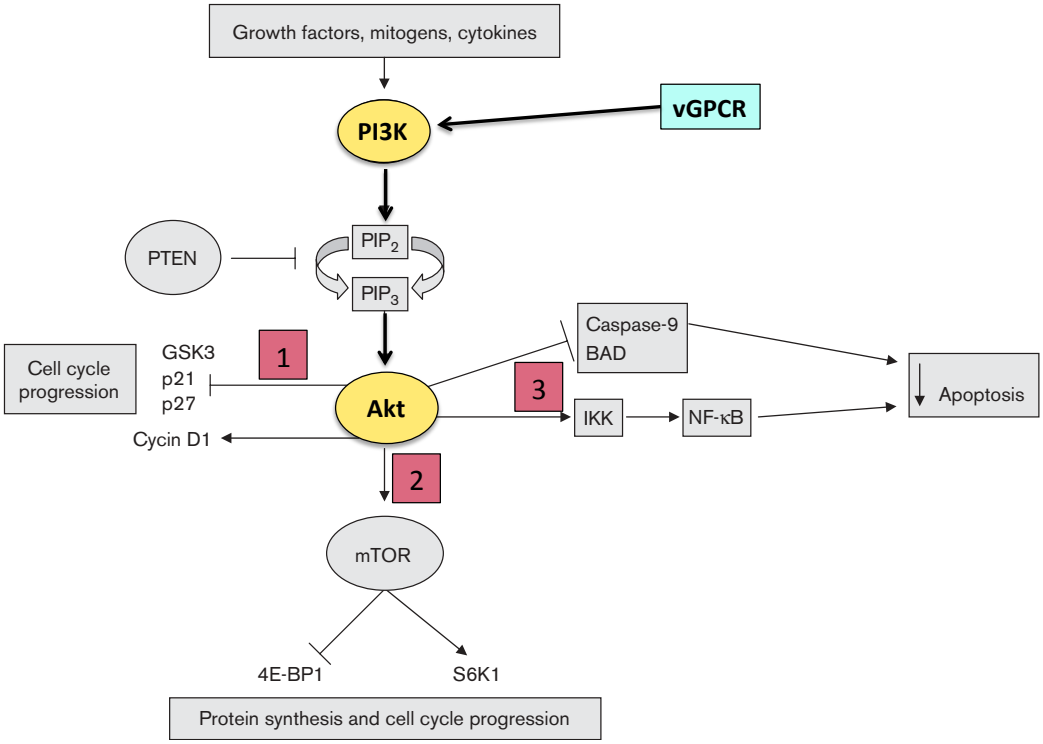

Figure 5: Schematic overview of the signaling cascade of the PI3K/Akt pathway. PI3K can be activated by diverse extracellular signals, including growth factors, or vGPCR as indicated in the figure. Consequently, activated PI3K acts as a lipid kinase and adds a phosphate group to PI-4,5 biphosphate (PIP_2) thereby transforming this protein into PI-3,4,5-triphosphate (PIP_3). Upon binding to PIP_3, Akt undergoes conformation changes resulting in activation and translocation to the cytosol and nucleus where Akt phosphorylates its substrates. Activated Akt regulated three main pathways, indicated by 1-3 in the figure. Pathway 1 leads to evading of growth suppression by inhibition of p21, p27 and GSK3 and activation of cyclin D. Pathway 2 leads to the promoting of cell growth via activation of mTOR and S6K1 and inhibition of 4E-BP1. And pathway 3 leads to inhibition of apoptosis via activation of NF-κB and inhibition of caspase 9 and BAD. Details about pathways 1 and 3 will be described in chapters 6.2 and 6.3 respectively.[86]

Abbreviations: PTEN, phosphatase and tensin homologue deleted on chromosome 10; Akt, also termed PKB (protein kinase B); mTOR, mammalian target of rapamycin; 4E-BP1, 4E-binding protein 1; S6K1, a protein serine/threonine kinase; GSK3, Glycogen synthase kinase 3, a protein serine/threonine kinase; BAD, Bcl-2-associated death promoter, a pro-apoptotic protein.

6.1.3 Alterations of the intracellular pathways responding to growth-stimulatory factors

The intracellular pathways are often altered in cancer. Factors in growth-promoting pathways are often overexpressed leading to an increased transcription of growth proliferation genes. Alterations in the intracellular pathways are more difficult to describe since growth stimulation can activate a variety of intracellular pathways. These pathways in turn cross-interact with other intracellular cascades as displayed in the intracellular circuit (Figure 3). Similar extracellular signals are also capable of inducing multiple pathways, and each pathway can cause diverse cellular biological effects such as proliferation, cell cycle activation, differentiation, apoptosis inhibition, and immune evasion. Which of these biological functions happen after activation of a specific pathway depends on so many cellular factors that it is often impossible to research one specific pathway on its own. The major proliferation pathways and factors that are often modified in cancer cells are the RAS/MAPK pathway and transcription factors c-Myc and NF-κB.[53,57]

KSHV LANA-1 stabilizes c-Myc

The transcription factor c-Myc is very often deregulated in various malignancies as it is an important player in cellular proliferation.[89] In BL c-Myc is translocated to the immunoglobulin loci thereby activating B cell proliferation.[90,91] Also in 16% of diffuse large B lymphomas c-Myc is amplified.[92] Fujimuro *et al*. reported in 2003 that KSHV encoded LANA-1 is able to promotes stabilization of β-catenin in PEL cells by inhibiting its negative regulator, GSK-3β.[93] As GSK-3β is also involved in the phosphorylation and thereby degradation of c-Myc Bubman *et al*. investigated a potential modulation of c-Myc in cells expressing LANA-1. They indeed concluded that PEL cells have abnormally stable c-Myc proteins, because LANA-1 inhibits GSK-3β-mediated phosphorylation of the T58 residue of c-Myc.[94]

Table 10

EBV and KSHV genes contributing to self-sufficiency in growth signals

	EBV	KSHV
Autocrine/paracrine growth factor	-	**vIL-6**
↑ Cytokine stabilization	-	**Kaposin B**
Activates JAK/STAT pathway and, AP-1 and NF-κB transcription	**LMP-1**	-
↑ EGFR expression	**LMP-1**	-
Activate PI3K/AKT pathway	-	**vGPCR**
Stabilize c-Myc	-	**LANA-1**

Blue text, latent gene; red text lytic gene

6.2 Evading growth suppressors

The majority of the cells in the human body are not continuously dividing but in a non-dividing quiescence state, implying that cell proliferation inhibitors exist. As cancer cells continuous proliferate they must have mechanisms to escape the anti-proliferative effect.[57]

6.2.1 The Retinoblastoma protein and its function in the cell cycle

Anti-proliferative signals influence the cell division cycle. Key regulators of this cycle are the tumor suppressor retinoblastoma protein (pRb), its two relatives p107 and p130, and the regulated cellular transcription factor E2F. Hypophosphorylated pRb forms a complex with E2F resulting in cell cycle arrest. If pRb is phosphorylated by the serine/threonine cyclin-dependent kinases (CDKs) E2F will induce cell cycle progression. To be able to function CDKs need to bind to cyclin (CYC) subunits, a process that can be inhibited by the cell cycle inhibitory proteins p15, p16, p21, and p27 (figure 3). In summary the cell proliferation status is dependent on the balance between the pro-cell division factors E2F, CDKs, and CYCs and anti-cell division factors pRb, p15, p16, p21, and p27. Soluble extracellular anti-growth factors, like TGFβ, and the tumor suppressor p53 can influence this balance by activating the anti-cell division proteins p15, p21, and p27 as indicated in figure 3.[57]

6.2.2 EBVs mechanisms to evade growth suppression

EBNA-3C deactivates pRb

Parker *et al*. indicated with co-transfection *in vitro* experiments that EBNA-3C can co-operate with activated Hras resulting in immortalization and transformation of rat embryo fibroblasts. This effect was not inhibited by co-transfection with the cell cycle inhibitor p16, but transformed cells are very susceptible to apoptotic cell death. *In vitro* direct binding of EBNA-3C to pRb was shown. This indicates that EBNA-3C can induce cell cycle progression through the inhibition of pRb by direct binding.[95]

6.2.3 KSHVs mechanisms to evade growth suppression

Both LANA-1 and vCyclin deactivate pRb

Immunoprecipitation assays in fibroblasts transfected with LANA-1 indicate that LANA-1 directly binds pRb thereby inhibiting pRb function. The cell cycle progression consequence of this binding was supported by a co-expression experiment with pRb negative cells. If these cells are transfected with both LANA-1 and pRb, LANA-1 is capable to inhibit the pRb induced cell cycle arrest in a dose-dependent manner.[96]
KSHV also encodes vCyclin, a homologue of human cyclin D2, one of the pro-cell cycle CYCs. *In vitro* studies indicate that vCyclin forms active kinase complexes with CDK6, resulting in the phosphorylation of pRb.[26,97] Unlike its cellular counterpart vCyclin seems resistant to p16, p21, and p27, making this protein a stronger pRb inhibitor.[98,99]

vGPCR evades growth suppression via the PI3K/Akt pathway

As described and shown in figure 5, vGPCR activates the PI3K/Akt pathway. This leads *inter alia* to the inhibition of the growth suppressors p21, p27, and GSK3 (pathway 1). This pathway also activates the growth stimulating cyclin D.

Table 11

EBV and KSHV genes contributing to evading growth suppressors

	EBV	KSHV
Deactivation of pRb	**EBNA-3C**	**LANA-1, vCyclin**
Deactivation of p21, p27, and GSK3 and activation of cyclin D via PI3K/Akt pathway		**vGPCR**

Blue text, latent gene; red text, lytic gene

6.3 Resisting cell death

As both virus infected cells as well as malignant cells can be recognized by the immune system, eradication of these cells is a major risk for their maintenance. A very important process in cell eradication is apoptosis. Apoptosis or regulated cell death can be considered as cell suicide without the attraction of inflammatory factors. For both cancer and viral persistence apoptosis must be effectively inhibited, and therefore most cancers and viruses have developed multiple apoptosis evasion mechanisms. In addition, EBV also has developed a mechanism to promote the survival of its main target cells: B cells.

6.3.1 The two routes leading to apoptosis

Apoptosis can be induced via two major signaling cascades: the intrinsic and the extrinsic pathway as shown in figure 6. A very important apoptosis inducing protein is protein 53 (p53). This so-called 'guardian of the genome' can induce apoptosis, in response to substantial levels of DNA breaks and other chromosomal abnormalities. P53 is often mutated in cancer and inhibited by viral proteins.[100,101]

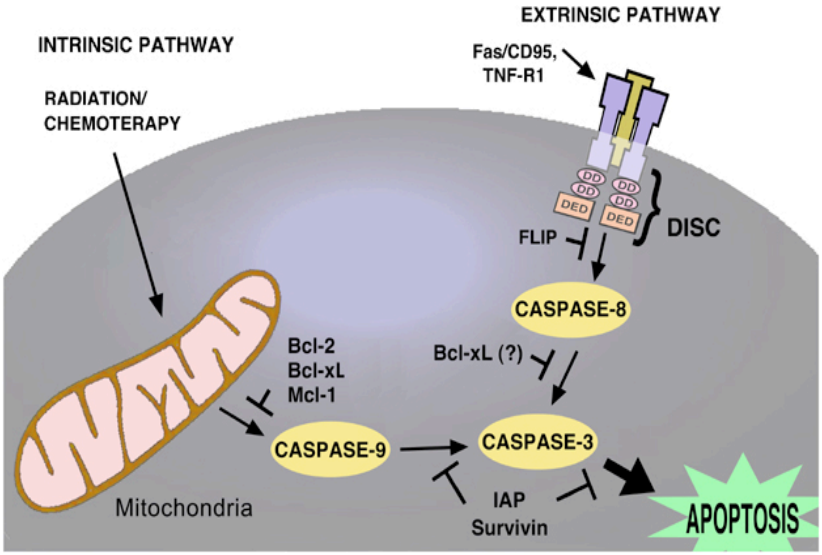

Figure 6: A simplified diagram of the two major signaling cascades that induce apoptosis: the intrinsic and the extrinsic pathway. The intrinsic pathway is triggered by intracellular abnormalities like DNA damage, signaling imbalance, insufficient survival signals or hypoxia.[102] Radiation, chemotherapy and p53 can also start the intrinsic pathway. Al these apoptotic triggers will change the balance of the regulators of the intrinsic pathway: proteins of the Bcl-2 family. Although members of this family contain the same functional domains, (BH) their function can be pro-apoptotic (Bax, BAD, Bak, Bok etc.) or anti-apoptotic (Bcl-2, Bcl-xL, Bcl-w, Mcl-1, etc). The Bcl-2 family members regulate the permeability of the mitochondrial membrane and thereby cytochrome c release from the mitochondria. Pro-apoptotic signals will result in cytochrome c release and thereby activation of caspase 9. Activated caspase 9 will activate the apoptotic caspase 3, a process that can be inhibited by IAPs as for example survivin.[103] Extracellular signals such as toxins, hormones, growth factors, nitric oxide and cytokines on the other hand, activate the extrinsic signaling pathway. The extracellular signals are transduced to the intracellular apoptotic members via death receptors such as Fas and TNF receptors.[104] Activation of these receptors leads to activation of their cytoplasmic DISC domain and thereby the activation of caspase 8 as indicated in the figure. This process can be inhibited by intracellular FLIP. Activated caspase 8 will activate caspase 3, which will finally result in apoptosis of the cell.[103]
Abbreviations: Caspase, cysteine aspartate-specific protease; IAP, inhibitors of apoptosis; DISC, death-inducing signaling complex; FLIP, FLICE-like inhibitory protein.

6.3.2 The cell survival mechanisms of EBV

LMP-1 inhibits apoptosis by various mechanisms

The EBV encoded latent LMP-1 protein can provoke resistance to apoptosis by diverse mechanisms. First, as described in detail above and in figure 4, LMP-1 is capable of activating, in a ligand-independent way, various pathways and transcription factors, including the JAK/STAT and JNK pathways and the AP-1 and NF-κB transcription factors. The final outcome of this activation can also result in the inhibition of apoptosis.[67] Although the precise mechanism by which NF-κB suppresses apoptosis is unknown, various *in vitro* and *in vivo* experiments have shown an important role for NF-κB in apoptosis inhibition.[105-107]

Secondly, LMP-1 is able to upregulate the expression of the anti-apoptotic protein Bcl-2. Henderson and colleagues showed that in a panel of EBV-positive BL cell lines, expressing the latency I phenotype, large numbers of cells went into apoptosis if the growth conditions were suboptimal (0,1% FCS). On the other hand, EBV-positive BL cell lines expressing latency III did not suffer from high numbers of apoptotic cells under the same conditions. Immunodetection of Bcl-2, with monoclonal antibodies, indicates that Bcl-2 was only expressed in latency III, possibly protecting these cells from apoptosis. To investigate which individual EBV latent gene can upregulate Bcl-2, expression vectors containing EBNA-1, EBNA-2, EBNA-LP, LMP-1, or LMP-2 were transfected in EBV-negative BJAB lymphoma cells. Immunodetection revealed that only the LMP-1 transfectant induced Bcl-2 levels and consequently reduced apoptosis, induced by suboptimal growth conditions, in the transfected cells The molecular mechanism of how LMP-1 upregulates Bcl-2 has yet to be elucidated.[108]

Thirdly, LMP-1 is capable of increasing the expression of the anti-apoptotic protein Mcl-1. Transfection studies with LMP-1 encoding plasmids in BL cell lines demonstrated that LMP-1 induced a rapid and transient upregulation of the Mcl-1 protein before the induction of Bcl-2.[109]

Fourthly, LMP-1 also increases the anti-apoptotic survivin. Ai *et al*. showed that the expression of survivin in LMP-1 positive NPC epithelial cells is higher than in LMP-1 negative NPC epithelial cells and that survivin is expressed in a LMP-1 dosage-dependent manner.[110]

The fifth anti-apoptotic mechanism of LMP-1 is based on the activation of the PI3K/Akt pathway. As indicated in figure 5 Akt activation can lead to an inhibition of apoptosis via NF-κB induction and caspase 9 and BAD reduction. Dawson and co-workers have shown that treatment of LMP1-expressing cells with a PI3K inhibitor results in decreased cell survival, thereby supporting the LMP-1 and PI3K/Akt pathway connection.[111]

The sixth and final known anti-apoptotic function of LMP-1 involves p53 inhibition. Okan and colleagues indicate with *in vitro* experiments that LMP-1 prevents p53-induced apoptosis but does not interfere with the p53-induced cell cycle arrest.[112] Liu *et al*. used LMP-1 deletion mutants and a p53 transactivation luciferase reporter assay, to demonstrate *in vitro* that the LMP-1 cytoplasmic tail domains, CTAR1 and CTAR2 are responsible for the p53 inhibition. As both these regions are also responsible for NF-κB activation a direct link between NF-κB activation and p53 inhibition was suggested. *In vitro* blockade of NF-κB indeed showed a reactivation of p53, thereby supporting that NF-κB activation also plays a role in p53 functional inhibition.[113]

Altogether LMP-1 has many different routes for apoptosis inhibition, indicating an important survival role for this protein in EBV infected cells.

EBNA-1 increases expression of the anti-apoptotic survivin

Although Henderson *et al*. concluded that EBV infected cells with latency I phenotype, thus expressing solely latent EBNA-1, were insufficient to inhibit apoptosis, as described in detail above, Lu and colleagues very recently indicated an apoptosis inhibitory role for EBNA-1. Using gene array data of EBV-negative BL cell lines, EBNA-1 transfected cells showed a 3.3 fold upregulated mRNA level of survivin compared to the control cut-off level. Western blotting and RT-PCR support this finding. In addition sections of diffuse large B cell lymphoma tissues, positive or negative for EBV, were stained by immunohistochemistry, which showed that expression of survivin overlapped with the expression of EBNA-1. *In vitro* inhibition studies with small hairpin RNAs (shRNA), showed that both survivin and EBNA-1 expression levels were knocked down in cells infected with survivin or EBNA-1 specific shRNA lentivirus indicating the direct connection between survivin and EBNA-1. In cells with knocked down survivin apoptosis levels were also higher than in the control cells. The effect on apoptosis in cells with knocked down EBNA-1 on the other hand, was less than the cells with knocked down survivin, indicating that EBNA-1 is not the only regulator of survivin.[114]

LMP-2A stimulates B cell survival

The B cell receptor (BCR) is a transmembrane protein located on the cell surface of B cells. The binding site of this receptor is able to bind antigens, which results in the activation of the intracellular signal cascade that leads to the differentiation of B cells into antibody-secreting plasma cells as shown in figure 7. Of importance is the non-proliferation or tonic signal, produced during this process, which is essential for B cell survival. LMP-2A, a latent 12 transmembrane EBV protein, is capable of mimicking this BCR cascade as shown in figure 7. As LMP-2A is able to induce the tonic survival signal it stimulates B cell survival.[67]

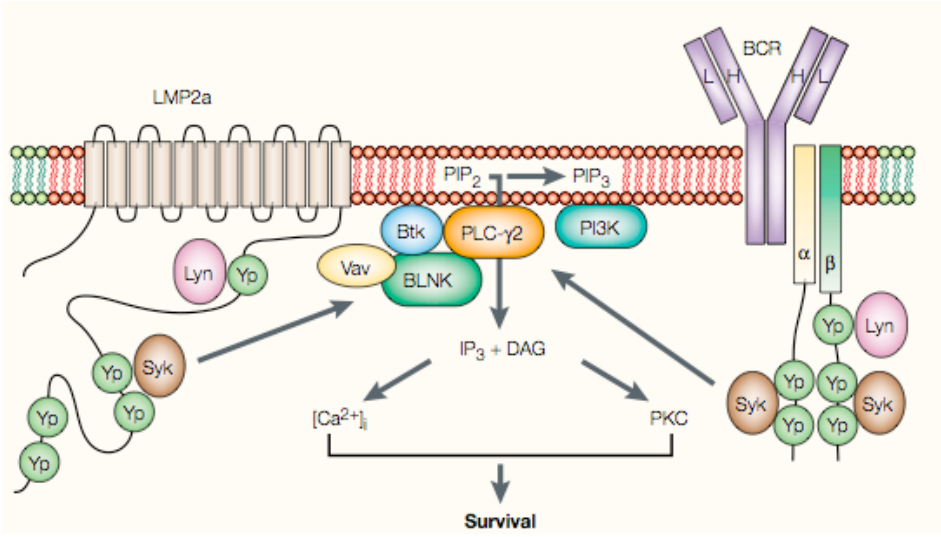

Figure 7: Schematic overview of the signaling relationship between LMP-2A and BCR. The amino-terminal domain of LMP-2A contains the same immunoreceptor tyrosine-based activation motifs (ITAMs) as found in the α- and β-chains of the BCR. Both intracellular ITAM domains of LMP-2A and BCR are associated with Lyn, a member of the Src family of tyrosine kinases. Phosphorylation of tyrosine residues within the ITAM by Lyn leads to recruitment of the Syk tyrosine kinase and thereby activation of the phospholipase. Both BCR and LMP-2A do not stimulate B cell proliferation but promote B cell survival.[67]
Abbreviations: BLNK, B-cell linker protein; DAG, diacylglycerol; [Ca2+]i, intracellular Ca^{2+}; PLC-γ2; phospholipase C-γ2; PKC, protein kinase C; Yp, phoshorylated tyrosine.

EBV BHRF1, a stronger apoptosis inhibitor than its Bcl-2 homologue

The lytic BHRF1 protein that is encoded by EBV is a homologue of the human anti-apoptotic Bcl-2 protein, which can inhibit the intrinsic activation of caspase 9 as described above. *In vitro* gene transfer studies have shown that BHRF1 can block apoptosis induced by withdrawal of serum, withdrawal of growth factors, treatment with the DNA-damaging anticancer agents cisplatin, etoposide and mitomycin C or infection with Sindbis virus.[115-117] Sindbis virus is known to induce apoptosis of infected cells.[118] *In vitro* experiments have indicated that some caspases, mainly caspase 3, are capable of converting the anti-apoptotic Bcl-2 and Bcl-xL proteins in death effector proteins by cleavage.[119] Interestingly BHFR1 is resistant to this caspase-mediated conversion and therefore a stronger inhibitor of apoptosis.[120]

6.3.3 The apoptosis inhibitor mechanisms of KSHV

Unlike EBV, KSHV has a wider variety of proteins involved in the inhibition of apoptosis.

Both LANA-1 and LANA-2 inhibit the pro-apoptotic function of p53

Because p53 is a very important protein in cell survival regulation, p53 is very often mutated in cancer. As p53 is not mutated in KS, this indicates a p53 inhibitory function for at least one of the KSHV encoding proteins.[121] Indeed Friborg *et al*. showed a direct binding of KSHV encoded latent LANA-1 to p53. In the lysates of KSHV-infected PEL cells and LANA-1 and p53-cotransfected p53-null Saos-2 osteosarcoma cells, immunoprecipitations of p53 with LANA-1 indicated their direct binding. In addition, transfection of LANA-1 significantly reduced the percentage of apoptotic cells, although the p53 levels in sample and control cells were comparable. This indicates that LANA-1 inhibits the apoptotic function of p53 by direct binding.[122]
In addition to LANA-1, LANA-2 is also associated with p53 inhibition. LANA-2 transfection *in vitro* inhibited 87% of p53 specific pG13-Luciferase reporter activity. In addition LANA-2 transfection in the wild-type p53 expressing U2OS cells, which were pretreated with the p53 activating chemotherapeutic Doxorubicin, led to a 57% inhibition of this p53 specific activation. Rivas *et al*. were however unable to demonstrate direct LANA-2-p53 interaction via immunoprecipitation leaving further support for the LANA-2 mechanism to be elucidated.[123]

vBcl-2, a very strong anti-apoptotic factor

Like EBV's BHRF1, the lytic KSHV encoded Bcl-2 (vBcl-2) is a homologue of the human anti-apoptotic Bcl-2 protein. As effective as cellular Bcl-2 and the related Bcl-xL proteins, vBcl-2 blocks apoptosis in diverse cellular systems.[124,125] Similar to BHRF1, vBcl-2 is resistant to caspase-mediated conversion into a pro-apoptotic protein.[120] In addition Bcl-2 family members regularly heterodimerize with other family members. This may result in a negative regulation of the anti-apoptotic activity of Bcl-2 and Bcl-xL if this heterodimerization occurs with pro-apoptotic members like Bax or Bak. However, vBcl-2 is unable to heterodimerize with other Bcl-2 family members, suggesting an evolved escape mechanism to negative regulatory effects.[125] All these characteristics make vBcl-2 a very dominant anti-apoptotic factor.

vFlip inhibits caspase 8 function and induces NF-κB activation

As indicated in figure 6 human cellular FLIP is capable of inhibiting apoptosis by the blockade of the extrinsic route via reducing caspase 8 activity. FLIP has a high structural homology to caspase 8 but lacks catalytic activity. Binding of FLIP to the death effector domains (DED) of death receptors like TNF therefore inhibits caspase 8 in a competitive manner.[126] Reduced levels of apoptotic cells in vFLIP transfected B cells compared with control cells, after treatment with apoptosis inducing soluble FAS, indicates an apoptosis protective role for vFLIP. In addition vFLIP induction in immunocompetent mice rapidly results in the development of aggressive tumors, supporting the anti-apoptosic function of vFLIP *in vivo*.[127] Besides caspase inhibition vFLIP is also capable of increasing cellular NF-κB levels *in vitro* by increasing phosphorylation of the NF-κB inhibitor IκB proteins.[128] Immunoprecipitation revealed that vFlip binds directly to the IκB kinases (IKK), resulting in activation of this latter. IKK proteins in turn phosphorylate IκB protein, thereby inducing NF-κB activation.[129] As described above the link between NF-κB activation and cell survival is supported by a lot of different *in vitro* and *in vivo* experiments. In summary vFLIP can promote cell survival in a dual manner: through the inhibition of caspase 8 and the activation of NF-κB.

vIAP increases cytosolic calcium levels to stabilize mitochondria

In 2002 two research groups identified another anti-apoptotic protein in the KSHV genome. This protein is encoded by K7 and named viral IAP (vIAP). Northern blot and RT-PCR studies of PEL cells showed vIAP expression exclusively in a lytic state. *In vitro* experiments indicate that vIAP protects cells from apoptotic stimuli such as TNF-α and anti-FAS monoclonal antibodies. Wang *et al*. also indicate localization to the mitochondria and the endoplasmic reticulum (ER), but were unable to unravel the anti-apoptotic mechanism.[130] Feng and co-workers on the other hand indicate that vIAP targets cellular calcium-modulating cyclophilin ligand (CAML) resulting in an increased cytosolic calcium (Ca^{2+}) concentration by enhanced ER Ca^{2+} release and activation of the capacitive Ca^{2+} pathway. In this way vIAP alters the kinetics and amplitudes of cellular Ca^{2+} responses on apoptotic stress, which regulates the organelle Ca^{2+} concentration. As a consequence, vIAP protects cells from mitochondrial damage and apoptosis.[131]

vGPCR inhibits caspase 9 and BAD and activates NF-κB via the PI3K/Akt pathway

As described and shown in figure 5, vGPCR activates the PI3K/Akt pathway. This leads *inter alia* to the inhibition of the apoptotic caspase 9 and BAD. Also NF-κB expression is stimulated via activation of IKK.

Table 12

EBV and KSHV genes contributing to cell death-resistance

	EBV	KSHV
↑ NF-κB reduced apoptosis	**LMP-1**	**vFlip, vGPCR**
↑ Anti-apoptotic Bcl-2	**LMP-1, BHRF1**	**vBcl-2**
↑ Anti-apoptotic Mcl-1	**LMP-1**	-
↑ Anti-apoptotic survivin	**LMP-1, EBNA-1**	-
↑ Anti-apoptotic IAP	-	**vIAP**
↑ PI3K/Akt apoptosis inhibition	**LMP-1**	-
↓ Caspase-8 activity	-	**vFlip**
↓ Caspase-9 activity	-	**vGPCR**
↓ Apoptotic BAD	-	**vGPCR**
Inhibition of P53	**LMP-1**	**LANA-1, LANA-2**
↑ B cell survival signal	**LMP-2A**	-

Blue text, latent gene; red text, lytic gene

6.4 Inducing angiogenesis

6.4.1 The angiogenic balance in human cells

Every cell in the human body needs uptake of oxygen and nutrients and removal of metabolic wastes and carbon dioxide to survive and therefore cells need to be located within 100 μm of a vascular vessel. To meet this requirement rapidly dividing cells, like cancer cells, need production of new blood vessels, a process termed angiogenesis.[132] Angiogenesis is determined by the balance between pro- and anti-angiogenesis regulators which bind to transmembrane tyrosine kinase receptors thereby activating different signal-transduction routes as the PI3K, AKT, p38 MAPK and Ras/Raf pathways.[133] The best-known angiogenesis promoting soluble factors are vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and hepatocyte growth factor (HGF). Other pro-angiogenic factors are angiotropin, angiogenin, epidermal growth factor (EGF), granulocyte colony-stimulating factor, IL-1, IL-6, IL-8, platelet-derived growth factor (PDGF), and TNF-α.[134-136]
Multiple viral proteins encoded by EBV or KSHV are capable of shifting the angiogenesis balance in favor of inducing angiogenesis, thereby possibly contributing to oncogenesis.

6.4.2 KSHV-induced angiogenesis

DiMaio and Lagunoff published a review in 2012 in which the angiogenesis promoting functions of various KSHV proteins are described. Based on both i*n vitro* and *in vivo* studies the angiogenesis molecular mechanisms of the proteins are reviewed. Based on this paper, angiogenesis stimulating functions of KSHV proteins are summarized in table 13. Important pro-angiogenesis factors that are induced by different KSHV proteins are hypoxia induced factor (HIF)-1α, VEGF-A and angiopoietin 1 and 4.[137] Many different KSHV proteins induce in particular VEGF-a, indicating the potential important function of this factor in the KSHV pathogenesis. HIF-1α is a transcription factor, important for upregulation of VEGF-A and stabilized during hypoxia.[138,139]
Thrombospondin-1 (THBS1) is an angiogenesis inhibitor protein that *inter alia* activates the anti-angiogenesis transforming growth factor-β (TGF-β) and is down regulated in KS. THBS1 is targeted by diverse KSHV miRNAs resulting in a decreased protein level.[140] In addition lytic vMIPs, encoded by KSHV, are chemokine-like factors that activate endothelial cells and stimulate angiogenesis.[141,142]
As Kaposin B stabilizes cytokine expression it can contribute to angiogenesis by increasing IL-6 and IL-8 levels. Kaposin B inhibits degradation of AU-rich cytokine mRNA by binding and activating MK2 kinase.[64] IL-6 and IL-8 are known to have angiogenesis stimulating capacity in some tissue but the molecular mechanism of IL-8 remains to be elucidated.[134,143] IL-6 is known to bind the IL-6 receptor gp130, thereby activating the intracellular JAK/STAT signal transduction pathway.[144] STAT3 plays a central role in signal transmission to the cell nucleus and based on *in vitro* and *in vivo* studies, Wei *et al*. concluded that IL-6 can induce VEGF via the STAT3 pathway without a hypoxic state.[145] As KSHV vIL-6 is capable of inducing the expression of human IL-6, this suggest an even higher level of IL-6 and therefore more VEGF induction.[63]

6.4.3 Mechanisms induced by EBV to stimulate angiogenesis

In EBV positive GC specimens the expression of both bFGF and VEGF angiogenesis stimulating factors is more abundant.[146] Unlike KSHV however there are only two EBV encoding proteins associated with angiogenesis stimulating capacity: LMP-1 and EBNA-1.[147,148] LMP-1 can induce the production of pro-angiogenesis factors IL-6, IL-8, HIF-1α, and VEGF in EBV-negative nasopharyngeal epithelial cells as *in vitro* studies show an increased level of these factors when LMP-1 is transfected in these cells.[147] Also EBNA-1 is capable of inducing HIF-1α, IL-8, and VEGF.[148] Wakischa *et al*. claim that NF-κB is involved in the LMP-1 pro-angiogenesis function and O'Neil and colleagues in contrast suggest the transcription factor AP-1 to be responsible for the angiogenesis-promoting role of EBNA-1. Interestingly, both studies were performed with transfection experiments in the same EBV-negative human nasopharyngeal cell line AD/AH.[147,148] Possible both transcription factors play a role in the transcription processes, as also indicating in figure 4 LMP-1 is at least able to activate both NF-κB and AP-1.[67]

Table 13
EBV and KSHV genes contributing to the induction of angiogenesis

	EBV	KSHV
↑ HIF-1α	**LMP-1, EBNA-1**	**LANA-1, LANA-2, vGPCR**
↑ VEGF-A secretion	**LMP-1, EBNA-1**	**K8.1, K1, vIL-6, vGPCR, Glycoprotein B, vMIP-I**
↓ Thrombospondin-1	-	**miRNAs**
Chemoattraction	-	**vMIPs I-III**
↑ Angiopoietin-1 secretion	-	**vIL-6, vGPCR**
↑ Angiopoietin-4 secretion	-	**vGPCR**
↑ IL-6	**LMP-1**	**vIL-6, Kaposin B**
↑ IL-8	**LMP-1, EBNA-1**	**Kaposin B**
↓ PECAM	-	**kK5**

Blue text, latent gene; red text, lytic gene

7. Evasion of immune eradication by EBV- and KSHV-associated malignancies

The human immune system is often referred to as the guardian of the body. It protects the body from extrinsic (harmful) molecules and pathogens by detecting and eliminating these intruders and destroying infected cells. In order to inhibit total eradication, both cancer and viruses need to prevent an immune response. Herpesviruses have evolved several effective strategies to avoid a host immune response and also the associated malignancies have mechanisms to avoid immune recognition. Both the viral as well as the malignancy mechanisms to evade immune recognition will be discussed in this chapter.

7.1 Viral antigen detection in EBV- and KSHV-associated malignancies

Although most cancers originate from cellular tissue, various research groups have indicated the existence of tumor antigens (TA), which can provoke an immune response to the cancer cells. Tumor antigens can consist of Tumor-Specific Antigens (TSAs), which are present exclusively on tumor cells, or Tumor-Associated Antigens (TAAs), which are present on both tumors and healthy cells, but can for example be amplified and/or overexpressed on tumor cells. TSA can occur through mutations in proteins, like the often-mutated p53 and RAS proteins in cancers.[149]
However, during the maturation of T cells, the cells that are able of strongly recognizing self-proteins are deleted. For that reason recognition of TSA or TAA by T cells is often inefficient and results in a weak response. In contrast, viral antigens present in EBV- and KSHV-associated tumors act as TSAs that can be recognized very efficiently by T cells, resulting in a strong immune response. *Inter alia* HL, NPC and KS are heavily infiltrated with T cells and studies on HL and NPC biopsies revealed that this T cell response is specific for EBV proteins.[13] Therefore immune recognition of EBV- and KSHV-associated malignancies is mainly based on the viral antigens they present.

7.2 Immune response against viral infection

The human immune system senses the presence of EBV and KSHV particles via a range of pattern-recognition receptors (PRRs) that recognize EBV- and KSHV-specific proteins and nucleic acids.[150] Detection of these so called pathogen-associated molecular patterns (PAMPs) activates all members of the innate immune system.[151] Components of the innate immune system can activate cells of the adaptive immune system: the T and B cells. Both subtypes of T cells, cytotoxic T cells (CTLs) and T helper cells, need T-cell co-stimulatory molecules as well as the detection of virally derived peptides on the surface of the virus-infected antigen-presenting cell (APC) to get activated. Many EBV and KSHV immune evasion strategies are based on inhibition of pathways involved in processing and presenting viral peptides, thereby avoiding T cell responses to virus infected cells.[152]

7.3 EBV and KSHV adaptive immune evasion mechanisms

MHC antigen presentation pathways

Major histocompatibility complex (MHC) antigen presentation pathways, involved in processing and presenting of viral peptides, are required to initiate T cell responses. Two subtypes of MHC exist: class I (MHC I) and class II (MHC II). Each MHC subtype is involved in a

distinguished antigen presentation pathway and results in the activation of CTLs and T helper cells for MHC I and II respectively.
In the MHC I antigen presentation pathway viral proteins are degraded by the cellular proteasome to short (8-11 amino acids) peptides in the cytosol of virus-infected cells. Next, transporter associated with antigen presentation (TAP) transports these viral peptides to the ER were they are bound to the host cell MHC I molecules. Subsequently, the MHC I:peptide complex is expressed on the cell surface, where CTL recognition induces apoptotic cell death. The CTLs also release immune stimulating cytokines. The importance of CTLs in eliminating EBV- and KSHV-infected cells is supported by the fact that these two viruses have diverse evasion mechanisms to every step in the MHC I antigen presentation pathway.[152]
In the MHC II antigen presentation pathway the viral antigens are obtained extracellular. Via endocytic uptake and degradation these antigens are converted into small peptides, ready to bind cellular MHC II. The MHC II pathway is therefore independent of proteasome activity. MHC II:peptide complexes are also expressed on the cell surface of APCs, which results in the activation of T helper cells. Activated T helper cells can in turn transform B cells into antibody secreting plasma cells and secrete cytokines that contribute to the activation of CTLs, thereby contributing to an immune response.

EBV and KSHV evasion mechanisms for the MHC class I antigen presentation pathway

The first viral mechanism to inhibit the MHC I antigen presentation pathway is the prevention of viral protein degradation to short peptides by the proteasome. Both EBV and KSHV encode for a latent protein, EBNA-1 and LANA-1 respectively, that is able to inhibit protein proteolysis. It was demonstrated by an *in vitro* translation assay, using an EBNA-1 protein (EBNA1ΔGA) lacking its internal Glycin–Alanine repetitive sequence (GAr) in comparison to wild-type EBNA-1 (EBNA1wt), that GAr causes inhibition of EBNA-1 proteolysis. In addition GAr insertion to the EBNA-3B (EBNA-4) protein resulted in the inhibition of CTL recognition of this chimeric EBNA-3B protein supporting the inhibitory role of GAr on proteasomal degradation.[153] LANA-1 contains a strongly acidic-repeat region that does not have sequence similarity with GAr. However, when using GFP as a marker intact LANA-1 showed no proteasomal degradation *in vitro* in contrast to the LANA-1 mutant lacking its acidic-repeat, indicating the same proteolysis inhibitory role for this region as GAr.[154]
Second, in addition to its role in the inhibition of EBNA-1 proteolysis, GAr is also capable of reducing mRNA translation of EBNA-1. Yin *et al*. found that EBNA-1 protein levels were increased in cells that express EBNA1ΔGA relative to cells expressing EBNA1wt. As GAr inhibits EBNA-1 degradation without affecting the mRNA levels of EBNA-1, the translation of both EBNA1ΔGA and EBNA1wt *in vitro* was studied. Results indicate a less efficient translation of the EBNA1wt transcript compared to that of EBNA1ΔGA by a factor 11.[155]
A third evasion mechanism is based on inhibition of TAP-mediated transport of the viral peptides into the ER. To date no KSHV protein is found to inhibit this process. In EBV on the other hand the BNLF2a protein blocks the TAP function by inhibiting the interaction of TAP with both the short peptide fragments as well as ATP. The latter is necessary as energy supply for translocation and opening of transmembrane pores. Inhibiting the transport process inhibits loading of the viral peptides on the MHC I molecule as this only takes place in the ER.[156]
Forth, both EBV and KSHV can decrease host cell protein synthesis including MHC I production. In both viruses an alkanine exonuclease (AE), named SOX in KSHV[157] and BGLF5

in EBV[158] has been shown to shut off the host cell protein production. Genes that are downregulated by SOX and BGLF5 encode *inter alia* MHC class I and II molecules, proteins that are very important for activation of both CTLs and T helper cells.[158]
Fifth, both EBV and KSHV can decrease the amount of cell surface expressed MHC I proteins via increased endocytosis and lysosomal degradation of these molecules. In EBV BILF1[159] and in KSHV the MARCH E3 ubiquitin ligases kK3 and kK5[160] (also known as MIR1 and MIR2 respectively[161]) are responsible for this function. kK3 facilitates the polyubiquitination of the MHC I cytoplasmic tail, which leads to endocytosis and lysosomal degradation of the MHC I molecule.[162] EBV BILF1 is also capable of reducing the newly synthesized MHC I:peptide complexes on their way to the cell surface, resulting in an even higher degradation of cell surface MHC I molecules.[163]

EBV evasion mechanisms for the MHC class II antigen presentation pathway

EBV encodes two lytic proteins, glycoprotein (Gp)42 or BZLF2 and BZLF1, which are capable of interfering with the MHC II pathway, resulting in an impaired T helper recognition of EBV-infected cells.
Ressing and colleagues determined crystal structures for gp42 and human leukocyte antigen (HLA)-DR1 (a MHC II protein in humans) complexes as well as T cell receptor (TCR)-MHC class II complexes, which show that gp42 sterically hinders TCR-MHC II interactions.[164]
More recently, the immediate-early lytic EBV gene BZLF1 was reported to interfere with MHC II antigen presentation. Li *et al*. described that expression of BZLF1 in the EBV-carrying B cell line Raji inhibited the expression of MHC II molecules. However, it cannot be ruled out that BZLF1, which also functions as a transcription factor promoting lytic translation, stimulates the expression of EBV lytic proteins in these cells. The MHC II downregulation may for that reason also be induced by another protein.[165]
In additional research, Zuo and co-workers indicate that BZLF1 downregulates CD74, thereby inhibiting T helper cell recognition of the virus-infected cell. EBV-negative MJS melanoma cells expressing MHC-II and EBNA-1, were transfected with plasmid vectors for BZLF1 or other EBV lytic genes. T helper cell activity against the EBNA-1 antigen was measured by the release of IFN-γ from the T helper cell. The BLZF1 expressing cells showed impaired EBNA-1 recognition by T helper cells. This impaired function did not correlate with reduced levels of surface MHC-II molecules, but rather with a marked downregulation of CD74 on the surface of target cells. As CD74 facilitates correct loading of antigenic peptide fragments onto the cellular MHC II complexes in the endolysosomal vesicles, downregulation of CD74 leads to impaired and non-immunogenic MHC II molecules.[166] Downregulation of CD74, however also leads to a reduction of the anti-apoptotic Bcl-2 family members and therefore to increased cell death. In an early stage of lytic infection this cell death can be overcome by expression of the lytic EBV BHRF1, a Bcl-2 homologue described in section 6.3.2.[166]

7.4 EBV and KSHV innate immune evasion mechanisms

Evading Natural Killer responses

NK cells continuously detect cell surfaces where both inhibitory and activating molecules for NK cells are expressed. Depending on the balance between these two, NK cells are activated or stay in a non-active state. Expressed MHC I molecules are the most abundant inhibitory ligands for NK cell receptors. Activation of the NK cells on the other hand happens through

cell surface activation ligands such as MICA, MICB, AICL, *etc*.[167] If EBV and KSHV are successful in the down regulation of MHC I molecules, as described above, they need some kind of NK evading mechanism to compensate for the lost in NK inhibitory signal.
KSHV's kK5 is known to inhibit a lot of different NK stimulating molecules via the stimulation of endocytosis and lysosomal degradation as described above. NK stimulating ligands, capable in binding the NKG2P and NKp80 receptors on NK cells and downregulated by kK5 are ICAM-I, B7-2, MICA, MICB, and AICL.[167,168] In addition both EBV and KSHV contain various microRNAs (miRNAs), miR-BART2-5p in EBV and miR-K12-7 in KSHV that also downregulate the NK stimulating MICB.[169] The fact that MICB is downregulated by so many different viral factors implies that MICB plays an important role in the immune destruction of EBV and KSHV infected cells.

INF signaling inhibition by KSHV

In human cells interferon (IFN) signaling is subdivided in type I and II. IFN type I contain two members, IFN-α and IFN-β, and is involved in constituting the primary response against viral infection. IFN type II contains one member, IFN-γ, and activation can lead to stimulation of the earliest immune responses, which is associated with the activation of NK cells and macrophages as well as the upregulation of cell surface expressed MHC I proteins. IFN-γ signaling also induces the formation of the active immunoproteasome from the dormant proteasome, resulting in an increase of the quantity, quality, and repertoire of viral peptides for the MHC I processing pathway.[170]
KSHV immediate-early nuclear transcription factor RTA is capable of decreasing IFN-α and IFN-β, by inhibiting interferon regulatory factor 7 (IRF-7), a key regulator of IFN type I induction. Co-transfection with RTA in mammalian cells blocked IRF7-mediated IFN-α and IFN-β mRNA production and promoted the ubiquitination and degradation of IRF7 protein in a proteasome-dependent way.[171] KSHV immediate-early protein, ORF45, also inhibits IRF-7 function. Zhu *et al*. found a direct interaction between the ORF45 protein en cellular IRF-7 by an *in vitro* binding assay. Co-transfection with ORF45 and mouse IRF-7 in HEK 293T cells, a human embryonic kidney cell line, indicate that ORF45 blocks IRF-7 phosphorylation and nuclear translocation, resulting in an efficient inhibition of type I IFN signaling.[172]
Li and colleagues examined the cell surface expression of IFN-γ receptor type 1 (IFN-γR1) by flow cytometry in cells transfected with KSHV encoded kK3 or kK5. Results showed that both kK3 and kK5 are able to induce the ubiquitination, endocytosis, and degradation of IFN-γR1, resulting in a down-regulation of the surface expression of IFN-γR1 and, thereby, inhibition of IFN-γ action.[173]

IL-10 signaling activation by EBV

Cellular IL-10 is an important regulator of immune and inflammatory responses in the human body. IL-10 can inhibit macrophage and T lymphocyte function by cytokine synthesis inhibitory factor (CSIF) activity, thereby inhibiting synthesis of cytokines important for the immune response such as IFN-γ, IL-2, IL-3, TNFα and GM-CSF. IL-10 also down regulates the expression of MHC II antigens, and co-stimulatory molecules on antigen presenting monocytes. On the other hand, IL-10 co-stimulates B lymphocyte proliferation, survival, and antibody production.
EBV encodes a lytic protein, BCRF1 that is homologous to human IL-10 and shares many of its functions. BCRF1 exhibits CSIF activity and also inhibits IFN-γ synthesis. In addition, BCRF1

is capable of co-stimulating B lymphocyte proliferation and differentiation. Altogether BCRF1 plays an important immune evasion. More details on IL-10 and BCRF1 can be found in the review of Moore *et al*. 1993.[174]

Reduction of TLR9 by EBV

One of the PRRs of the innate immune system capable of recognizing EBV molecules is toll-like receptor (TLR)9. The importance of this receptor in EBV immune recognition is supported by the inhibitory role of both the lytic BGLF5 as well as the latent LMP-1 EBV proteins towards TLR9. *In vitro* studies by van Gent *et al*. indicate that BGLF5 is able to downregulate the mRNA levels of TLR9 resulting in a decreased expression of the receptor on the cell surface.[175] Fathallah and co-workers support the inhibitory role of EBV on TLR9, but assign this function to the latent LMP-1 protein. Mutational inhibition of LMP-1 results in a loss of TLR9, indicating a prominent role for this protein. LMP-1 functions via NF-κB signaling as NF-κB inhibition also results in a loss of TLR9 inhibition.[176]

Table 14
EBV and KSHV genes contributing to immune surveillance evasion

	EBV	KSHV
↑ Resist proteolysis	**EBNA-1**	**LANA-1**
↓ Own mRNA translation	**EBNA-1**	-
↓ TAP-mediated transport viral proteins	**BNLF2a**	-
↓ MHC class I production	**BGLF5**	**SOX**
↑ MHC class I lysosomal degradation	**BILF1**	**kK3**, **kK5**
MHC class II steric hindrance	**Gp42 (BZLF2)**	-
↓ MHC class II viral antigen loading	**BZLF1**	-
↓ IFN-γ	**BCRF1**	**kK3**, **kK5**
↓ IFN-α and IFN-β	-	**RTA**, **ORF45**
↑ CSIF	**BCRF1**	-
↓ NK stimulating signaling	**miR-BART2-5p**	**kK5**, **miR-K12-7**
↓ TLR9	**BGLF5**, **LMP-1**	-

Blue text, latent gene; red text, lytic gene

7.5 Immunodominance of EBV-associated malignancies

T cells need *inter alia* the detection of virally derived peptides, presented in MHC I or II molecules, on the surface of APCs to get activated. CTLs recognize MHC I-peptide complexes and T helper cells detect MHC II-peptide complexes. Various studies have indicated that the efficiency of this T cell detection is variable per viral peptide and also the efficiency by which antigens are presented on the surface of lytically infected cells is changeable. Both variables lead to the concept of hierarchies of immunodominance among viral proteins, indicating that some viral peptides provoke a stronger immune response than others.[177 177]
Various methods have been designed to determine the immunodominance of viral proteins and in 2007 Hislop *et al*. summarized the immunodominance of EBV proteins tested until then. Their results are shown in figure 8. Based on this information it is known that EBNA-3 A-C are immunodominant in CTL response and that on the other hand EBNA-1, EBNA-2 and EBNA-3C are immunodominant in T helper response. LMP-1 is not immunogenic in both CTL and T helper cell responses.[177]

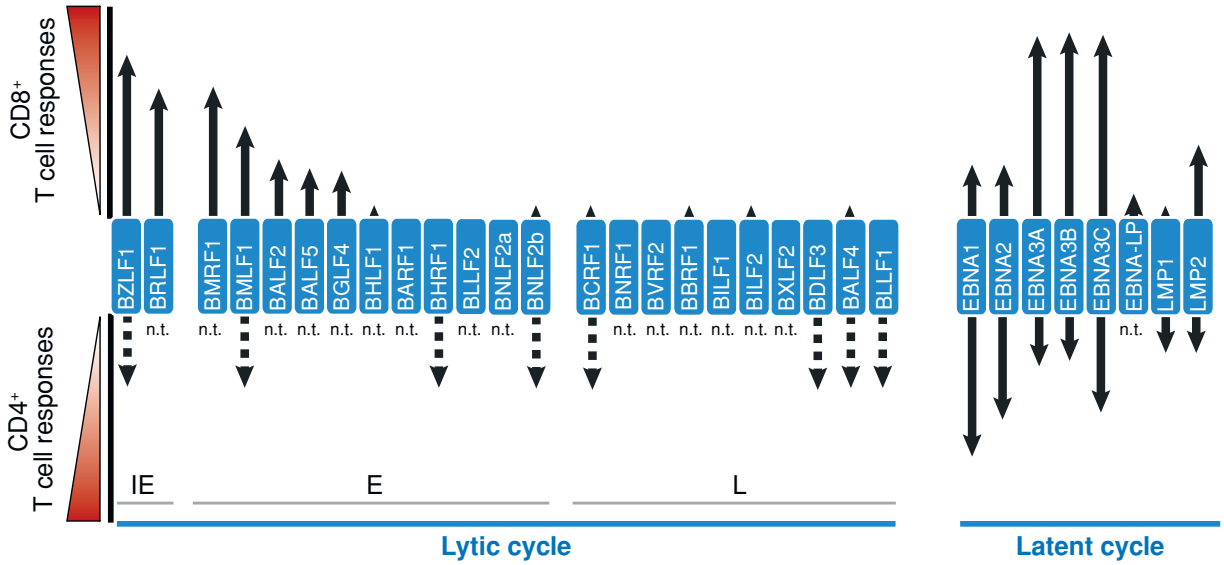

Figure 8: Diagrammatic representation of the relative immunodominance in healthy virus carriers of both lytic and latent proteins for CTL and T helper cell responses. Dotted arrows denote that T helper cell responses have been observed for these proteins but that their relative immunodominance is not yet determined. Proteins not tested are denoted as n.t.[177]
Abbreviations: CD8+, CTL; CD4+, T helper cell

Based on the information in figure 8 and the gene expression patterns of EBV-associated malignancies as shown in table 8, CTL and T helper cell immune response of EBV-associated malignancies can be determined. Of interest is the low CTL response against EBNA-1 as a result of EBNA-1's capacity to resist its own proteasomal degradation and to decrease its own mRNA translation as described in detail in section 7.3. Furthermore Münz and Moormann summarized all known CTL and T helper responses against EBV related malignancies as indicated in figure 9.[178] Briefly, PTLD and HIV-associated diffuse large cell lymphoma induce a broad immune response of both CTL and T helper cells, via all eight EBV latent proteins. As the immune system is deficient, by immunosuppressive drugs or HIV, in these malignancies, virus-infected cells will not be eradicated despite this broad immunogenic expression pattern. HL, on the other hand, is less immunogenic and induces dominant T helper response via EBNA-1 and a subdominant CTL response via LMP-2 protein expression. BL is the least immunogenic malignancy as it only expresses EBNA-1, which in turn induces dominant T helper en subdominant CTL responses.

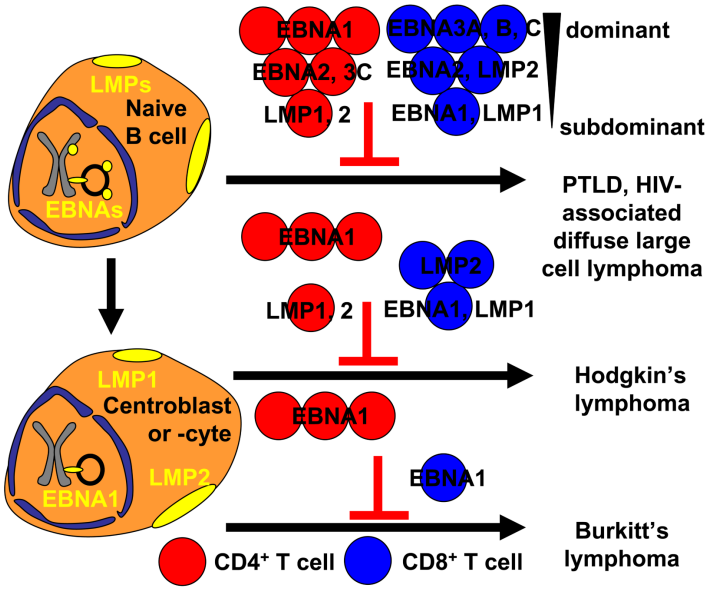

Figure 9: EBV-associated lymphomas vary in the viral antigens they express as well as the B cell differentiation stage of origin. As a result the immune response against these lymphomas varies in dominance as indicated in the figure.[178]

7.6 Immunodominance of KSHV-associated malignancies

Robey *et al* summarized known T cell epitopes derived from KSHV genes in 2011 and found some CTL epitopes derived from LANA-1, LANA-2 and Kaposin B. CTL and T helper responses preferentially target early and late lytic KSHV gene products. As the epitope determination for KSHV is still an evolving field, the dominant or subdominant states of KSHV proteins have not been determined yet.[179]

7.7 Immune evasion by EBV-associated malignancies

Limited latent antigen expression leads to a reduction in immunogenicity and is thereby the most essential immune escape mechanism of EBV- and KSHV-associated malignancies. In addition some EBV-associated malignancies are able to induce local immunosuppression, thereby promoting their persistence and growth. Two studies have indicated the presence of regulatory T cells (Treg) in NPC tissue. As Tregs are involved in immunosuppression, by *inter alia* secretion of TGF-β and IL-10, homing of these cells to the tumor site can provide local immunodeficiency.[180,181] In addition various studies have indicated that HRS cells in HL are capable of producing the immunosuppressive cytokines IL-10, IL-13, and TGF-β.[182-184] The presence of Tregs in HL tissue is also reported.[185] In conclusion, both NPC and HL seem to have the ability to downregulate immune response against tumor tissue on a microenvironmental basis, thereby contributing to oncogenesis and tumor persistence.

8. Discussion

The strong association between EBV or KSHV and specific malignancies is generally accepted. However, the long list of barriers for determining an etiologic link between a virus and cancer, as listed in table 2, shows the difficulty of distinguishing between an associative versus causative relationship. In tables 3-5, different sets of guidelines of establishing causality are listed and when these guidelines were compared minimal criteria, present in all three sets, were extracted. These criteria are the epidemiologic association between the oncovirus and its related cancers, both in geographical distribution as well as in viral markers present in the tumor, and the capacity of promoting growth of the virus *in vitro* at the minimum.

In chapters 3 and 4 the association between EBV or KSHV and their related malignancies is described and it is shown that the association between EBV or KSHV and their related malignancies is very variable and difficult to interpret. The oncogenic capacity of EBV and KSHV, however, is easier to determine. As described in detail in chapter 6, both viruses contain various genes with oncogenesis stimulating functions and mechanisms. Several, but not all, of these genes are expressed in the associated malignancies, as is summarized below in tables 16 and 17. Based on this data it becomes clear that the genomes of both EBV and KSHV have the capacity to stimulate a range of cancer hallmarks: (I) cell growth stimulation, (II) growth suppression evasion, (III) resisting cell death, and (IV) induction of angiogenesis. In EBV the latent EBNA-1 and LMP-1 primarily provide these oncogenic capacities, whereas in KSHV these capacities are provided by both latent LANA-1 and lytic vGPCR. In addition many other latent and lytic KSHV genes have oncogenic capacity as shown by the long list in table 17. Altogether this indicates that both viruses have sufficient oncogenic capacity in their genome to be able to cause cancer.

In this review the proliferative capacity of both EBV and KSHV *in vitro* as well as *in vivo* is not described, as this has already been extensively covered by the review of Pagano and co-workers.[4] In this paper a causal relation between an oncovirus and its related malignancies

Table 15

Infectious Agent	Malignancy	Transforms Human Target Cells	Tumors in Animal Model	Present in Tumor Tissue %	Cofactors	Epidemiology	Causative
EBV	BL	+	-	15 – 100[a]	Malaria	+	No[b]
EBV	HL	+	-	20 – 80[a]	?	+	No
EBV	NPC	+	+	30 – 100[a]	Genetic, environ.	+	Yes
EBV	PTLD	+	+	80 – 100[a]	Immunodef.	ND	Yes
EBV	GC	+	-	15 – 100[a]	?	ND	No
KSHV	KS	+	?[c]	100	Immunodef, genetic	+	Yes
KSHV	PEL	-	-	100	Immunodef.	ND	Yes
KSHV	MCD	?	?	40 – 100[a]	Immunodef.	ND	Yes

EBV and KSHV and their associated malignancies and oncogenic properties[4,7]

[a] Depending on subtype as indicated in tables 6 and 7

[b] EBV is cofactor

[c] The related Simian radinovirus induced tumors in animal models

abbreviation: ND, not done

for *inter alia* EBV and KSHV is established by the minimal criteria described above. Based on *in vitro* and *in vivo* transformation by the virus, epidemiologic evidence and the association between the virus and the malignancy, Pagano *et al*. determined viral causality. Their findings are presented in table 15. In this same table the findings of another paper, in which Damiana reviewed the causal relation between DNA tumor viruses and human cancer, are also included.[7] Based on their data KSHV can be considered etiologic in KS, PEL and MCD, whereas EBV can only be considered etiologic in NPC and PTLD and not in BL, HD and GC. EBV is considered a cofactor in BL, which is consistent with the fact that BL does not express LMP-1 and therefore lacks the capacity to stimulate cell growth and avoid growth suppression.

Despite this conclusion there are still some reservations about the etiologic roles of EBV and KSHV in their related malignancies. The use of the minimal criteria to distinguish between a causative or associative role may not give the complete picture.

Pagano *et al*. are hesitant about the etiologic role of EBV in NPC because environmental and/or genetic cofactors have also been strongly linked to oncogenesis in this malignancy. In addition they list the presence of EBV in NPC as 100% in their paper, whereas Rickinson and Kieff claim the presence to be between 30-100% depending on the subtype (table 6). The combination of these two factors is a reason to question whether the relationship is truly causal.

The presence of EBV in BL is less than 100% in non-endemic subtypes, which is an indicator of an associative rather than a causative relationship. More importantly BL is characterized by specific translocations, which are absent in other EBV-associated malignancies. If EBV truly causes BL, it is expected that these translocations would be present in other EBV-infected cells as well. The inconsistent presence of EBV in HL tissues is also a reason to exclude EBV from its etiologic role in this malignancy. However, mechanisms have been proposed which are compatible with such an inconsistent presence, such as the hit and run model, which will be covered below.

The IARC, on the other hand, does consider EBV to be causative in BL and HL,[186] which demonstrates the difficulty in the determination of the etiological role and the large influence of the interpretation of the specific researcher.

In KSHV on the other hand the clonality of the KSHV-malignancies plays an important role in the determination of the causal role. The monoclonal status of the tumor indicates that the tumor has originated from one single virus infected cell and that viral infection has occurred prior to tumor development, thereby indicating an important role of the virus in the oncogenesis. If malignancies are polyclonal, however, multiple virus-infected cells are involved and viral infection is more likely to have occurred during oncogenesis. In this case the viral infection probably does not play a significant role in oncogenesis. As MCD is a polyclonal tumor the causality of KSHV is doubtful. KS tumors are often monoclonal but polyclonal tumors also occur, leading to the possibility that there are two different oncogenic mechanisms. In this case only one of the mechanisms involves KSHV infection, although clearly proving causality in such a case is challenging.

Another very important aspect in the determination of a causal role of EBV and KSHV in cancer that is also noted in the reviews of both Pagano and Damania is immune deficiency. It is a cofactor of at least PTLD, KS, PEL and MCD as shown in table 15. Remarkably the associated virus is also indicated as causal in these malignancies.

As is described in chapter 7 in detail, both EBV and KSHV have multiple mechanisms, performed by multiple genes (table 14), to escape immune evasion. These immune evasion mechanisms of EBV and KSHV genes are also included in tables 16 and 17. In addition the

EBV related malignancies NPC and HL have the capacity of repressing immune surveillance in their microenvironment, thereby contributing to immune evasion mechanisms.
However, despite all these immune evasion mechanisms, EBV- and KSHV-malignancies are still recognized by the immune system. As indicated in detail in paragraph 7.5 and figure 8 there are various T cell epitopes that have been determined. EBV epitopes are derived from all EBV latent proteins. On the other hand KSHV epitopes that have been identified thus far are derived from KSHV lytic proteins and a selective collection of KSHV latent proteins. As only limited research has been done on this topic, the expectation is that the amount of T cell epitopes from KSHV latent proteins will grow in the next couple of years.[179]
Additional support for immune recognition of EBV related malignancies is the presence of EBV specific CTLs, that are still functional after culture *in vitro*, from isolates of HL and NPC biopsies.[187,188] Also EBNA-1 specific T helper cells are capable of recognizing BL cells.[189,190]
Possibly due to their inability of avoiding immune recognition, most EBV- and KSHV-associated malignancies occur in immunocompromised or immunodeficient persons like severe combined immunodeficiency patients, organ-transplant recipients and patients with AIDS.[4]
In EBV related malignancies for example, two independent studies have found that selective loss of EBV specific T helper cell responses correlates with the development of EBV non-Hodgkin's lymphomas in HIV infected individuals.[191,192] Also T cell responses against EBNA-1, the only EBV protein expressed in the majority of BL cells, were significantly decreased in nearly all children with BL.[193] Additionally passive immunization in which autologous EBV specific T cells are co-stimulated with EBV transformed B cells *in vitro* and subsequently injected into PTLD patients, is used as a functional treatment for these patients.[194]
Also in KSHV related malignancies immune suppression plays an important role as *inter alia* indicated by a study of Guihot and colleagues where KSHV-specific T cell responses in KS patients were compared to asymptomatic KSHV carriers.[195] KS patients indeed showed a lower number of KSHV-specific T cells. In addition the incidence of KSHV positive PELs is lower in immunocompetent individuals compared to HIV infected patients.[178] Furthermore the use of HAART, the highly active antiretroviral therapy that is used to treat AIDS patients, has lower the incidence of AIDS-associated KS.[196] As HAART is associated with immune reconstitution to KSHV a direct link between a working immune response and the prevention of KS is made.[197] This same phenomenon is seen in iatrogenic KS patients when the immune response is restored due to withdrawal of immunosuppressive drugs.[198]
In summary, the development of EBV- and KSHV-associated malignancies can be seen as a multifactorial process in which infection by the virus is a possible requirement but immunosuppression is an important cofactor that cannot be neglected. KSHV and EBV infection alone does not seem to be sufficient to cause cancer and reversing immunosuppression even appears to lead to regression of the tumor.
Numerous research papers mention immune control as an important protection against the onset of malignancies. However few researchers speculate about the cellular molecular mechanisms that are affected by immune deficiency and ultimately lead to oncogenesis. Currently a lot of research is done to discover how oncoviruses and their associated malignancies escape immune control. But perhaps an even more interesting question is: what is changed in the virus-infected cells through immune deficiency that leads to cancer development? It seems very likely that there will be a change in the viral expression.
Sodhi and co-workers hypothesized a model in 2004,[16] which implies that deregulated expression and activity of vGPCR in a fraction of tumor cells is necessary and sufficient to initiate KS. Additionally an event must occur to trigger this vGPCR deregulation, which can be

immunosuppression. The consequence of this deregulation would be the existence of two sets of vGPCR-expressing cells: normally lytic infected cells and deregulated vGPCR-expressing cells that do not lyse. Although this model has not been proven yet, one important feature of this theory is very likely to be true: immunosuppression leads to viral gene deregulation. As a defect immune system results in the loss of control of viral-infected cells, possibly these cells react by changing the number of genes they express or their gene expression profile to a more immunogenic profile potential with lytic gene expression. However, lytic genes promote viral replication, ultimately resulting in cell lysis and therefore are often excluded to play a role in oncogenesis. It is however remarkable that 1-5% of the cells in a single KHSV tumor expresses lytic genes. For some lytic genes, like vIL-6 and vGPCR, this low percentage could be sufficient to play a role in oncogenesis, as their paracrine effects are able to affect the whole tumor. It is therefore possible that lytic genes do contribute to oncogenesis in such a manner.

Another possible explanation for the potential role of lytic genes in oncogenesis is the hit and run-oncogenesis model, established by Skinner in 1976.[199] This model states that viral infection can trigger or "hit" cellular transformation of infected cells into malignant cells through a misdirected immune response, mutagenic activity, or permanent chromatin reorganization. Additionally these mechanisms activate oncogenes or silence tumor suppressor and DNA repair genes, leading to oncogenesis. Subsequently maintenance of the malignant state is compatible with recombinogenic activities that may lead to loss or "run" of viral molecules. The hit-and-run model therefore raises the possibility of an etiological role for viruses in oncogenesis in malignancies that lack the expression of any viral genes and proteins. Niller *et al*. support the hit and run model by *in vitro* observations that confirm integration of the EBV genome into the cellular DNA and subsequent loss of this viral DNA in BL cells.[200]

In the case of EBV and KSHV possibly the loss of viral molecules is specific for the more immunogenic and lytic proteins. The maintenance of latent proteins benefits the malignancies, but lytic proteins eventually induce cell death and thus hinder the maintenance of malignancies. Removal of the lytic genes or inhibition of their expression would therefore be favorable for the malignancy once it has formed.

To obtain more insight in what is changing in the viral genome in malignant cells, future research can focus on gene expression profiles of organ transplant patients. A lot of these patients will develop PTLD, and examining the gene expression profiles during this process, for example every week, may be able to give new insights in which genes are important in oncogenesis.

In conclusion, EBV and KSHV are strongly associated with certain malignancies, but determining whether this association is etiologic is challenging. Based on minimal criteria for this determination, Pagano *et al*. defined causality and concluded KSHV causes KS, PEL and MCD and EBV exclusively causes NPC and PTLD, is a cofactor in BL and is only associated with BL, HD and GC. However, the important role of immune deficiency cannot be neglected, as KSHV and EBV infection alone do not seem to be sufficient to cause cancer. The development of EBV- and KSHV-associated malignancies can therefore been seen as a multifactorial process in which infection by the virus is a possible requirement but immunosuppression is an important cofactor. The mechanisms by which immune suppression contributes to oncogenesis are however unclear and further research is needed to elucidate these. This may hopefully contribute to developing specific treatments of EBV- and KSHV-associated tumors and help determine possible cancer prevention strategies.

Table 16

Oncogenesis stimulating functions and mechanisms of EBV genes

Gene product	Functions	Mechanism	1	2	3	4	5	Expression
LMP-1	Cell growth stimulation, Anti-apoptotic, Pro-angiogenesis Innate and CTL immune evasion	Activates JAK/STAT pathway, Increases EGFR, NF-κB, AP-1, Bcl-2, Mcl-1, survivin, PI3K, HIF-1, VEGF-A, IL-6 and IL-8, Inhibits p53, Resists self-proteasomal degradation and downregulates TLR9 expression	+	-	+	+	+	HL, NPC, PTLD, and NK/T L
LMP-2A	Promotes B cell survival	Activates B cell survival signal	-	-	+	-	-	HL, NPC, PTLD, and NK/T L
EBNA-1	Anti-apoptotic Pro-angiogenesis CTL Immune evasion	Increases HIF-1, VEGF-A, IL-8 and survivin, Resists self-proteasomal degradation, Decreases own mRNA translation	-	-	+	+	+	BL, HL, NPC, PTLD, PEL, and NK/T L
EBNA-3C	Evading growth suppression	Inhibits pRb	-	+	-	-	-	PTLD
miBART 2-5p	NK immune evasion	Downregulates NK stimulating MICB	-	-	-	-	+	HL, NPC, PTLD
BNLF2a	CTL Immune evasion	Downregulates TAP-mediated transport	-	-	-	-	+	
BGLF5	Innate and CTL immune evasion	Downregulates MHC I production, Downregulates TLR9 expression	-	-	-	-	+	
BILF1	CTL immune evasion	Upregulates lysosomal degradation of MHC I molecules	-	-	-	-	+	
Gp42 (BZLF2)	T helper immune evasion	Steric hindrance MHC II	-	-	-	-	+	
BZLF1	T helper immune evasion	Decreases peptide loading onto MHC II	-	-	-	-	+	
BCRF1	Immune evasion	Homologue of the immunosuppressive IL-10	-	-	-	-	+	

Blue text, latent gene; red text, lytic gene

1, cell growth stimulation; 2, growth suppression evasion; 3, resisting cell death; 4, induction of angiogenesis; 5, evasion of immune detection

NK/T L, NK and T cell lymphoma

Table 17

Oncogenesis stimulating functions and mechanisms of KSHV genes

Gene product	ORF	Functions	Mechanism	1	2	3	4	5	KS	PEL	MCD
LANA-1	73	Cell growth transformation, Anti-apoptotic, Pro-angiogenesis	Inhibits p53 and pRb, Increases HIF-1 and c-myc stability Resists self-proteasomal degradation	+	+	+	+	+	II	II	II
vFLIP	K13	Anti-apoptotic	Blocks caspase 8 activation, induces NF-κB activity	-	-	+	-	-	I	I	I
vCyclin		Evading growth suppression	Inhibits pRb	-	+	-	-	-	I	I	I
Kaposin B	K12	Cytokine promoting	Blocks cytokine mRNA degradation	+	-	-	+	-	I	II	?
miRNAs		NK immune evasion, Pro-angiogenesis	Downregulates MICB and thrombospondin-1	-	-	-	+	+	?	?	?
vIL-6	K2	Cell transformation, Pro-angiogenesis	Activates JAK/STAT and MAPK pathways, Increases VEGF-A and angiopoietin-1 secretion	+	-	-	+	-	III	III	III
vGPCR	74	Cell transformation, Pro-angiogenesis	Activates PI3K/AKT/mTOR pathway and NF-κB, AP-1, and HIF-1α transcription, Increases VEGF-A and angiopoietin-1/4 secretion	+	+	+	+	-	I	I	I
LANA-2 (vIRF3)	K10.5-10.6	Pro-angiogenesis, Anti-apoptotic	Increases HIF-1 stability, Inhibits p53	-	-	+	+	-	-	II	III
vBcl-2	16	Anti-apoptotic,	Inhibits pro-apoptotic Bcl-2 family proteins	-	-	+	-	-	?	?	?
vIAP	K7	Anti-apoptotic	Inhibits caspase 3 activation and function	-	-	+	-	-	?	?	?
kK3 (v-MIR1)	K3	IFN and CTL immune evasion	Downregulates IFNγRI, Stimulates MHC I lysosomal break-down	-	-	-	-	+	?	?	?
kK5 (v-MIR2)	K5	NK, IFN and CTL immune evasion, Pro-angiogenesis	Downregulates ICAM-I, B7-2, MICA/B MICB, AICL, IFNγRI, and PECAM, Stimulates MHC I lysosomal break-down	-	-	-	+	+	?	?	?
K8.1		Pro-angiogenesis	Increases VEGF-A secretion	-	-	-	+	-	?	?	?
K1		Pro-angiogenesis	Increases VEGF-A secretion	-	-	-	+	-	III	III	II
Gb		Pro-angiogenesis	Increases VEGF-A secretion	-	-	-	+	-	?	?	?
vMIP I-III	K3-4.1	Pro-angiogenesis	Chemoattraction	-	-	-	+	-	?	?	?
SOX		CTL immune evasion	Downregulates MHC class I production	-	-	-	-	+	?	?	?
RTA	50	IFN immune evasion	Inhibits IRF-7	-	-	-	-	+	I	I	?
ORF45	45	IFN immune evasion	Inhibits IRF-7	-	-	-	-	+	?	?	?

Blue text, latent gene; red text, lytic gene;

1, cell growth stimulation; 2, growth suppression evasion; 3, apoptosis inhibition; 4, resisting cell death; 5, evasion of immune detection

?, unknown; I, RNA; II, RNA and protein; III, RNA and protein but protein is only detected in small percentages of tumor cells

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