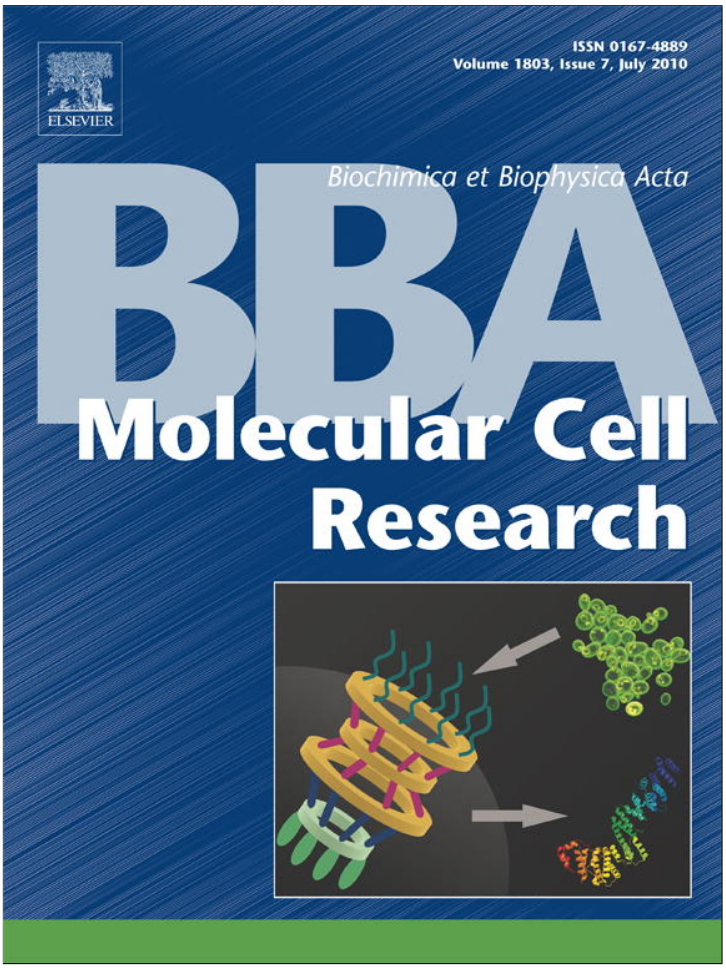

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Review

Herpesviruses: Hijacking the Ras signaling pathway

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ABSTRACT

Cancer is the final result of the accumulation of several genetic alterations occurring in a cell. Several herpesviruses and especially gamma-herpesviruses have played an important role in Cancer Biology, contributing significantly to our comprehension of cell signaling and growth control pathways which lead to malignancy. Unlike other infectious agents, herpesviruses persist in the host by establishing a latent infection, so that they can reactivate periodically. Interestingly, some herpesviruses are able to either deliver or induce the expression of cellular oncogenes. Such alterations can result in the derailment of the normal cell cycle and ultimately shift the balance between continuous proliferation and programmed cell death. Herpesvirus infection employs key molecules of cellular signaling cascades mostly to enhance viral replication. However, most of these molecules are also involved in essential cellular functions, such as proliferation, cellular differentiation and migration, as well as in DNA repair mechanisms. Ras proteins are key molecules that regulate a wide range of cellular functions, including differentiation, proliferation and cell survival. A broad field of medical research is currently focused on elucidating the role of *ras* oncogenes in human tumor initiation as well as tumor progression and metastasis. Upon activation, Ras proteins employ several downstream effector molecules such as phosphatidylinositol 3-kinase (PI3-K) and Raf and Ral guanine nucleotide-dissociation stimulators (RALGDS) to regulate a cascade of events ranging from cell proliferation and survival to apoptosis and cellular death. In this review, we give an overview of the impact that herpesvirus infection has on the host-cell Ras signaling pathway, providing an outline of their interactions with the key cascade molecules with which they associate. Several of these interactions of viral proteins with member of the Ras signaling pathway may be crucial in determining herpesviruses' oncogenic potential or their oncomodulatory behavior. The questions that emerge concern the potential role of these molecules as therapeutic targets both for viral infections and cancer. Understanding the means by which viruses may cause oncogenesis would therefore provide a deeper knowledge of the overall oncogenic process.

1. Introduction

1.1. Ras signaling pathway

Ras proteins are membrane-bound molecules that regulate a wide range of cellular functions, including differentiation, proliferation and cell survival [1]. A broad field of medical research is currently focused on elucidating the role of *ras* oncogenes in human tumor initiation as well as tumor progression and metastasis [2]. More specifically, Ras proteins are found to be mutated in many tumors such as melanoma, ovarian and lung carcinoma [3,4]. Genetic alterations of *ras* genes usually shift the balance between cell proliferation and cell death, towards continuing proliferation and differentiation. The role of *ras* genes during normal cellular function is dictated by the post-translational modification to which the proteins are subjected, which is mainly farnesylation. This process determines the final location of the proteins in the cell, primarily the plasma membrane, as well as their functionality. The enzyme farnesyl transferase links a farnesyl group (15-carbon isoprenoid) covalently to a cysteine residue located in the carboxy-terminal CAAX motif of Ras, allowing Ras to be

Abbreviations: BL, Burkitt's lymphoma; COX-2, cytochrome *c* oxidase subunit II; E, Early; EBNA, Epstein–Barr nuclear antigen; EBV, Epstein–Barr virus; EGF, Epidermal Growth Factor; EGFR, Epidermal Growth Factor Receptor; Elk, Ets-related transcription factor; ERK, extracellular regulated kinase; GDP, guanosine diphosphate; GTP, guanosine triphosphate; HCMV, Human Cytomegalovirus; HHV, Human Herpes Virus; HL, Hodgkin's lymphoma; HSV, Herpes Simplex Virus; IE, Immediate Early; IL, interleukin; JNK, c-Jun amino-terminal kinases; KSHV, Kaposi Sarcoma-associated Herpes Virus; L, Late; LAT, latency-associated transcript; LNA, latent nuclear antigen 1; MAPK, Mitogen-Activated Protein Kinases; MAPKK, MAPK kinase; MAPKKK, MAPK kinase kinase; MCD, multicentric variant of Castleman Disease; NF-κB, Nuclear Factor kappa-light-chain-enhancer of activated B cells; NGF, Neuronal Growth Factor; NPC, Nasopharyngeal Carcinoma; ORF, Open Reading Frame; PEL, Primary Effusion Lymphoma; PI3-K, phosphatidylinositol 3-kinase; PKR, RNA-activated protein kinase; RA, retinoic acid; RALGDS, Ral guanine nucleotide-dissociation stimulators; RTA, viral replication and transcriptional activation protein; TNF-α, tumor necrosis factor; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate; VZV, Varicella Zoster Virus

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anchored to the plasma membrane. It is known that the mis-positioned Ras proteins are non-functional, probably due to the inability of these proteins to employ their target enzymes and initiate signal transduction. Binding of ligands to tyrosine/kinase transmembrane receptors, autophosphorylates their tyrosine residues in the cytosol. The tyrosine residues serve as intracellular docking sites specific for several adaptor molecules such as members of the SOS family. Ras proteins remain inactive in the cell when they are bound to guanosine diphosphate (GDP), but become activated when bound to guanosine triphosphate (GTP) [5,6]. Ras is activated when ligand-bound receptors nucleate a complex including adapter molecules [e.g., Src homology and collagen (Shc; protein), Gab2, and growth factor receptor binding protein 2 (Grb2)], the phosphatase SHP-2, and guanine nucleotide exchange factors (e.g., SOS). Guanine nucleotide exchange factors bind to Ras and catalyze guanine nucleotide dissociation, which results in increased Ras-GTP levels. Ras activation is terminated by hydrolysis of GTP to GDP. This reaction is greatly accelerated by the GTPase-activating proteins p120GAP and neurofibromin.

Activation of Ras is responsible for the sequential phosphorylation of downstream molecules which amplify and transduce signals from the cell surface to the nucleus. More specifically, Ras proteins can employ up to 20 downstream effector molecules such as phosphatidylinositol 3-kinase (PI3-K) and Raf and Ral guanine nucleotide-dissociation stimulators (RALGDS) to regulate a cascade of events ranging from cell proliferation and survival to apoptosis and cellular death [7–12]. The activated Raf kinase, in particular, activates the MAPK cascade (Fig. 1). The MAPK cascade in the mammalian cell comprises three well-characterized protein kinases that are activated by protein phosphorylation: a MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK) and finally a MAPK. The final protein kinases (MAPKs) are the extracellular regulated kinase (ERK1/2), p38 kinases, ERK5 and the c-Jun amino-terminal kinases (JNK12/3). The Ras/Raf/MEK/ERK pathway is generally induced by cell surface receptors such as the Epidermal Growth Factor Receptor (EGFR), whereas the p38 and JNK kinases respond to stress signals as well as growth factor expression [13].

1.2. Herpesviruses and the Ras pathway

The Ras pathway molecules respond to various extracellular stimuli which eventually determine the fate of the cell. Environmental, chemical and infectious agents have the ability to affect the signaling process in the cell, altering its physiological function. Human herpesviruses are of particular interest, since they are able to either induce tumor initiation (oncogenic viruses), or regulate tumor behavior (oncomodulatory viruses). Interestingly, 17.8% of worldwide cancer cases are attributed to infectious agents, while 12.1% of the total cancer cases are caused by viral infections [14]. Essentially, the above 12.1% represents almost 70% of the total (17.8%) caused by infectious agents.

Bearing these epidemiological data in mind, as well as the causative role of herpesviruses in several human diseases, involving key signaling molecules of the host, we review this family of viruses focusing on the Ras signaling pathway. Eight human herpesviruses have been extensively studied and several of them have been associated with the oncogenic Ras signaling pathway. In more detail,

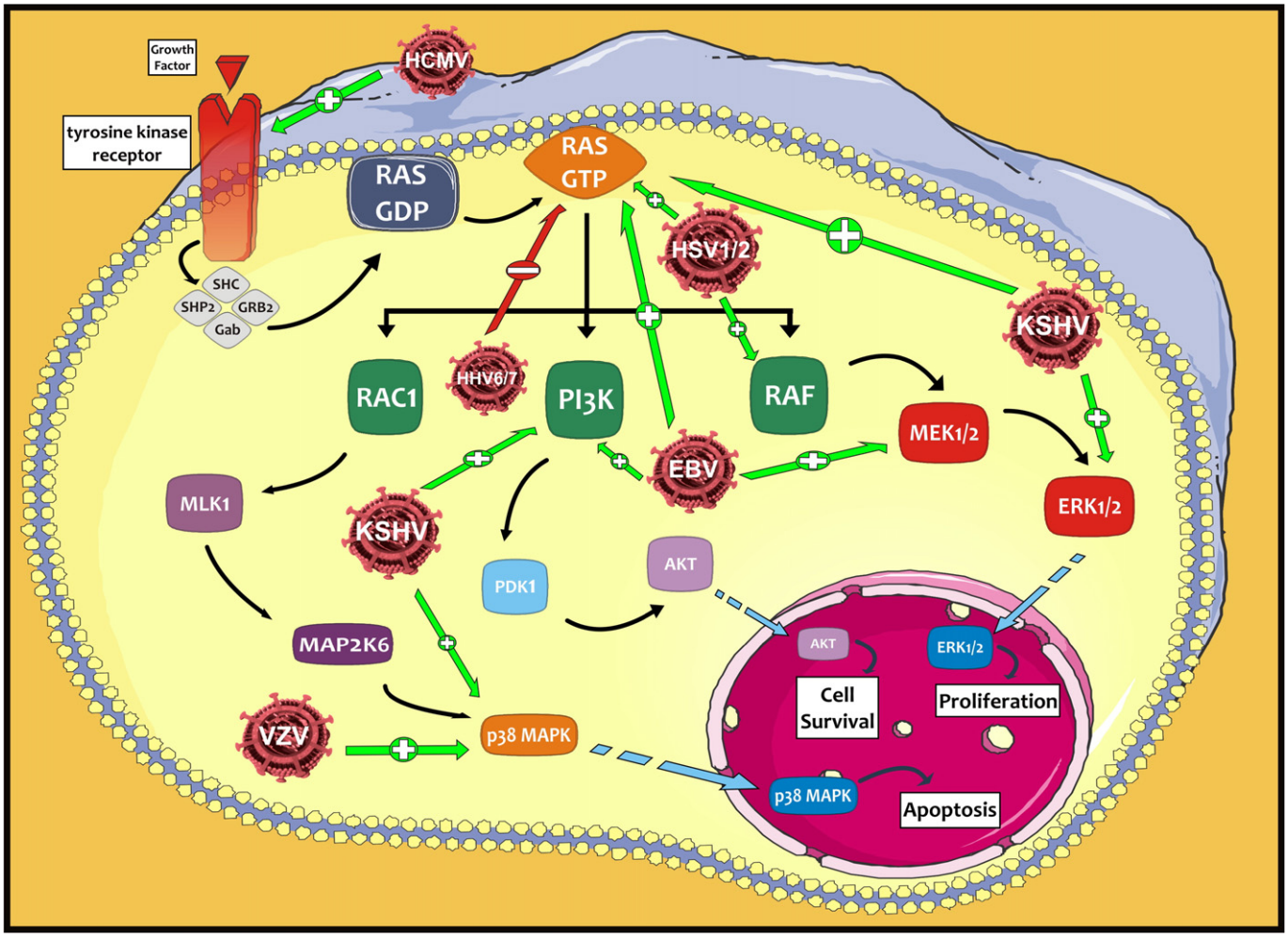

Fig. 1. Host cell Ras signaling pathway. The key mediators of the pathway are represented. Interactions between herpesviruses and host cell proteins are illustrated, indicating whether the virus up-, or down-regulates a specific cellular molecule.

the viruses are: Herpes Simplex Virus-1 (HSV-1 or HHV-1), Herpes Simplex Virus-2 (HSV-2 or HHV-2), Human Herpes Virus Type 3 (HHV-3) or Varicella Zoster Virus (VZV), Epstein–Barr virus (EBV or HHV-4), Human Cytomegalovirus (HCMV or HHV-5), Human Herpes Viruses -6 and -7 (HHV-6 and HHV-7) and finally the Kaposi Sarcoma-associated Herpes Virus (KSHV or HHV-8).

Upon infection of a host cell, a number of cellular defense mechanisms are employed to eliminate the threat, followed by acute inflammation. Although inflammation is generally considered to be beneficial for the cell, the environment of the inflammation site is a very potent place for transformations to occur. During inflammation a wide array of DNA-binding proteins, cytokines and chemokines are recruited to combat the inflammatory signal. TNF-α, NF-κB, IL-1, COX-2 and the pro-inflammatory chemokine families CXC and CC usually have positive effects on cell proliferation. However, their ability to cause DNA damage in the inflammation site has been shown to have a negative effect on normal cell proliferation and angiogenesis [15–18]. Herpesviruses possess the ability to evade the host immune response during initial exposure, and remain in a latent state until they reactivate to carry out lytic infection [19,20]. Additionally, some herpesviruses can promote oncogenesis either by delivering oncogenes in the host cell or by activating cellular oncogenes. The activation of oncogenes in the host cell leads to the expression of transforming growth factors that deregulate normal cellular functions and result in malignant cell transformation [21,22].

We provide an overview on the effect of herpesviral infection to the host-cell Ras signaling pathway, focusing on gamma-herpesviruses and their association with the key cascade molecules with which they interact.

2. Alpha-herpesviruses

Alpha-herpesviruses are the only subfamily of the herpesviridae family, known as neurotropic pathogens. Thus, HSV and VZV are able to infect and remain latent inside nerve cells and sensory ganglia. Upon reactivation, the two viruses trigger a painful localized *vesicular* rash. For HSV-1, it is usually lesions that form around the mouth area of the infected individual, whereas for HSV-2 the outbreak is known as genital herpes. VZV, on the other hand, is the causative agent of two distinct clinical diseases: primary infection that leads to Varicella (chickenpox) and reactivation from a latent state in sensory ganglia that gives rise to zoster (shingles).

2.1. Herpes Simplex Virus type-1 and type-2 (HSV-1 and HSV-2)

Productive infection by HSV-1 involves the timely expression of approximately 80 viral genes. These genes are expressed in three sequential phases: Immediate Early (IE), Early (E) and Late (L). Many products of these viral genes have been shown to interact with host encoded proteins during infection in order to ablate the host-cell defenses and finally replicate [23–27]. In addition, HSV-1 has the ability to overcome cellular defense mechanisms in tumor as well as in normal cells [28–32]. More specifically, experiments carried out on H-*ras* transformed cell lines have shown that HSV-1 replicates in these cells by inhibiting the virus-induced activation of the double-stranded RNA-activated protein kinase (PKR) [33]. Phosphorylation of PKR results in the phosphorylation of the translation initiation factor eIF-2α, resulting in inhibition of the viral transcripts in the host cell. PKR activation is essentially the major defense of the host cell against viral infections.

PKR is also impaired by the expression of the HSV-1 neurovirulence protein ICP34.5. ICP34.5 ablates host-cell PKR function by inducing the dephosphorylation of eIF2α [34,35]. Inhibition of PKR activity allows for the favorable expression of viral proteins, resulting in impaired host-cell defense. Furthermore, HSV-1 establishes a latent infection in sensory neurons in a Ras-dependent fashion [36]. Infection in these cells by HSV-1 is characterized by the over-expression of a series of transcripts, termed latency-associated transcripts (LATs). Both Ras and Raf molecules are activated in response to Neuronal Growth Factor (NGF) stimulation, which leads to the activation of LATs and the establishment of latent infection [37]. In lytically infected cells, both HSV-1 and HSV-2 possess an anti-apoptotic activity, as was previously described [38–40]. The two viruses inhibit apoptosis in a cell type-specific manner. This inhibition is attributed to the US3 gene, as well as to the US5 and ICP27 genes of HSV-1 [41,42]. More specifically, expression of the HSV-1 ICP27 protein results in the enhanced activation of the p38 and JNK cellular pathways [42]. Activation of the p38 pathway by ICP27 results in the induction of apoptosis, whereas activation of the JNK pathway by ICP27 requires the co-expression of one or more early or late viral proteins. ICP27 appears to play a role as a pro-apoptotic factor for the host cell since ICP27 expression alone cannot inhibit the apoptotic signaling that takes place after p38 activation [43]. Induction of apoptosis in cells infected by ICP27 mutant viruses, probably involves the destabilization of Bcl-2, which is mediated by p38/MAPK pathway activation. Bcl-2 loss of function is induced by p38 expression and results in sensitization and induction of apoptotic cell death processes [44]. Bcl-2 function in HSV-1 infected cells is crucial for the fate of the host cell, and may prove to be a key mediator molecule for HSV-1 infection.

Interestingly, the HSV-2 tegument protein ICP10 PK [45–47], appears to mediate activation of the host-cell Ras/Raf/MEK/ERK pathway. Indeed, the levels of activated MAPK1/2 significantly increased in hippocampal cultures infected by HSV-2 when compared to mock-infected cultures [48]. The inhibition of MAPKs by the chemical inhibitor UO126 caused a dose-dependent increase in apoptotic cells, suggesting that HSV-2 infection is actually mediated through the cellular MAPK pathway, as shown in Fig. 1 [49]. Hence, the infection of HSV-2 is mediated and enhanced by the host-cell *ras* pathway since the viral ICP10 PK activates Ras, MEK and MAPK while protecting the cell from apoptotic cell death [50,51].

Several studies have used mutant forms of HSV-1 and HSV-2 to target tumor cells [52,53]. The mutant HSV forms are known as oncolytic viruses since they are genetically modified to express their proteins and replicate in tumor but not in normal cells [53,54]. These conditionally replicating viruses infect and eventually kill tumor cells either by inducing apoptosis, or by destroying these cells with the cytolytic activity they possess [55,56]. For instance, the oncolytic G207 HSV-1 strain is able to achieve enhanced permissiveness to malignant peripheral nerve sheath tumors (MPNSTs). It is believed that over-activation of Ras in MPNSTs leads to increased viral replication in these cells and not in the normal *ras* expressing cells [57].

Wild-type HSV-2 on the other hand is able to selectively replicate and lyse tumor cells [58], such as neuroblastoma, renal, breast and pancreatic cancers [59–61]. The abovementioned ICP10 PK protein of HSV-2 is responsible for activating the Ras pathway leading to deregulation of the cell cycle and uncontrolled proliferation. Deletion of the PK subunit of the viral ICP10 protein impairs the ability of HSV-2 to infect normal cells that have an inactive Ras pathway [62]. Recent experimental data using recombinant Signal-Smart (SS1) HSV-1 viruses, show that upon exposure of ELK-overexpressing prostate cancer cells to the recombinant virus, the cells' proliferation, invasiveness and colony formation capabilities are significantly decreased, whereas the rate of apoptosis/necrosis functions is increased [63]. Additionally, increased Ras signaling cells exposed to the recombinant virus, showed a conspicuous G1 cell cycle arrest when compared to cells infected with parental HSV-1. Oncolytic HSV-1 viruses provide an excellent tool to study oncogenic signals and open novel paths in using these biological agents for detection and targeting cancer cells, with augmented specificity and efficiency.

2.2. Varicella Zoster Virus (VZV)

The Varicella Zoster Virus genome encodes approximately 70 gene products, expressed in a timely fashion similar to the expression of HSV proteins (IE, E and L). Lytic infection from the virus leads to Varicella, a clinical manifestation also known as chickenpox that includes symptoms such as fever and a generalized vesicular rash. Reactivation of the virus from its latent state usually occurs during adulthood and presents a distinct, localized vesicular rash that is uncommon in herpesviral infections [64].

As with all herpesvirus infections, VZV-induced infections are known to utilize host-cell signaling pathways in order to achieve increased permissiveness [65]. Upon infection, the cell responds by secreting, among others, interleukins (ILs). Interleukins and particularly IL-8, play a crucial role in the immune response of the host cell, since it acts as a key mediator during acute inflammation with a variety of actions on several tissues [66]. Secretion of IL-8 in VZV-infected T cells is dependent of the status of both the JNK/SAPK and p38/MAPK pathways. Hence, an active p38/MAPK pathway is required for IL-8 secretion and a successful immune response in T cells [67]. Moreover, infection by VZV leads to the activation of the MAPK pathway by a two-fold increase of p38/MAPK phosphorylation (Fig. 1), and ultimately to the deregulation of the normal cell cycle and proliferation processes [68–70]. Although there is a direct association of VZV infection with the MAPK pathway, it remains unclear whether the virus interacts with upstream molecules such as Ras, since current studies have merely focused on the phosphorylation levels of MAPK, as well as the characterization of two Open Reading Frames (ORF49 and ORF61) [71,72]. ORF61 encodes proteins with transregulatory activities such as the activation/phosphorylation of MAPKs in the host cell, whereas ORF49 is synthesized at late infection stages since it is part of the virion component.

3. Beta-herpesviruses

The beta-herpesviruses subfamily consists of the members HCMV, HHV-6 and HHV-7. In order to gain entry into the host cell, beta-herpesviruses, as with all herpesviruses, use virion envelope proteins to adhere to the cell membrane and depose the virion components into the cytoplasm. HCMV in particular, appears to infect a wide range of cell types, such as fibroblasts, endothelial cells, epithelial cells, monocytes/macrophages, smooth muscle cells, neuronal cells, neutrophils, stromal cells and hepatocytes [73,74]. The broad cellular tropism that HCMV demonstrates in the human body, justifies the increased manifestation of the virus in the worldwide population. On the other hand, HHV-6 and HHV-7 are restricted to T lymphocytes and certain cells of the myeloid lineage [69].

3.1. Human Cytomegalovirus (HCMV)

Human Cytomegalovirus is a widespread pathogen; an estimated 50–85% of the population worldwide is currently infected by HCMV [75]. In particular, HCMV is able to remain latent in the monocyte-macrophage cell lineage for extended periods of time, and is reactivated when the individual is immuno-compromised [76]. Data suggest that gene products of Human Cytomegalovirus can modulate tumor cell biology, promoting mutagenesis, cell cycle progression, angiogenesis, cell invasion and immune evasion [77,78].

There is increasing evidence which confirms the presence of HCMV in malignant tumors such as malignant glioblastoma [79,80], colon cancer [81], as well as EBV-negative Hodgkin's lymphoma [82]. Studies on human colonic adenocarcinoma cells (Caco-2 cells), have produced contradicting results. In one study the authors suggest that Human Cytomegalovirus infection can only arise when Caco-2 cells are in a specific state of differentiation, in which the virus does not spread from cell to cell, and productive infection is rare, whereas the other group supports that Caco-2 cells are infected by HCMV, regardless of the differentiation state [83,84]. In glioblastoma, the HCMV Immediate Early-1 (72 kDa) protein (IE1) is expressed in >90% of the tumors analyzed. A stable IE1 expression can differentially affect the growth of human glioblastoma cells, resulting in either growth proliferation or cell cycle arrest [85]. Given the high HCMV infection rate in these tumors, it can be hypothesized that a sustained expression of critical viral genes such as IE1 may have important biological consequences in malignant glioma cells. Moreover, these findings provide important insights into the pathogenic mechanisms associated with aberrant signaling pathways, transcription factors and/or tumor suppressor functions of the host cell.

Although HCMV is not directly associated with tumor formation, the timely expression of its proteins regulates the host-signaling pathways so that the virus can be sustained in the cell [86]. To accomplish this, HCMV has to bind to specific cellular receptors in order to initiate infection. Upon infection by HCMV, the viral particles must first enter the host cell and then travel to the nucleus in order to initiate viral replication. Interestingly, HCMV entry into the host cell is achieved by the synergistic action of the Epidermal Growth Factor Receptor (EGFR) and the activation of the β3 integrin subunit. In turn, this results in the activation of downstream molecules such as the focal adhesion kinase, PI3K/Akt and phospholipase Cγ [87–89]. However, it is also argued that EGFR is not required for the cellular expression of viral proteins or virus-induced signaling [90,91]. In addition to EGFR and integrin, PDGFα and β have also been proposed as cellular receptors for HCMV entry [92–94].

The binding of the viral particles to their respective receptors [95,96] initiates a cascade of events that permit virus entry and subsequent viral replication (Fig. 1). It is known that the Ras/Raf/MEK/ERK pathway is activated when the phosphorylation of EGFR occurs on the cell surface [97]. It is further believed that the gB glycoprotein of HCMV shares common sequences with the Epidermal Growth Factor (EGF), which acts as a ligand for EGFR [98,99], allowing infection to occur.

Initial studies have shown that the steady-state levels of *c-H-ras* increase in T2 cells upon RA-induced differentiation. Moreover, the expression of exogenous oncogenic human *H-ras* in T2 cells allows for HCMV infection, as well as changes in cell surface antigens observed in the early stages of RA-induced differentiation [100]. Our recent experimental data, obtained using H-*ras* transformed cells, further support the enhanced permissiveness of HCMV augmenting the progeny viral yield and the viral gene expression. Notably, HCMV infection resulted in a further increase of the proliferation rate, mobility and formation of cellular foci in the H-*ras*-activated cells compared to mock-infected cells or the non-transformed parental cell line (Filippakis, Spandidos, Sourvinos, unpublished data). These observations suggest that HCMV employs the oncogenic Ras pathway for the induction of cellular and/or viral gene expression and enhanced viral permissiveness. The communication between viral glycoproteins and cellular receptors is usually sufficient to induce activation of the ras pathway. However, there are several cellular responses that follow infection, including the phosphorylation of signaling kinases and the subsequent activation of transcription factors, cytokines and prostaglandins. Successful HCMV infection requires the activation of MEK1/2 and ERK1/2, two kinases that are crucial elements of the Ras pathway [101]. Inhibition of these cellular proteins by the inhibitors UO126 and PD098059 blocks the productive HCMV infection in the cell, suggesting that the two molecules are very important for virus replication [102]. The abovementioned virus-induced events impair the host-cell defense by interacting with molecules of the Ras signaling cascade, thereby rendering the cell more permissive to the virus. The relationship between HCMV and cancer has been in the center of virology research for decades. The frequent presence of a non oncogenic virus, such as HCMV, in malignant tissues results in increased proliferation of HCMV-infected

tumor cells when compared to the uninfected tumor cells. HCMV may infect tumor cells and modulate their proliferation and overall survival, without direct transformations taking place. These oncomodulatory effects of HCMV infection arise from the interactions between viral regulatory proteins and key signaling pathways such as the Ras/Raf/MEK/ERK, p38 and Akt pathway [103].

3.2. Human Herpes Virus 6 and 7 or Roseolovirus (HHV-6, HHV-7)

HHV-6, which belongs to the beta subfamily of herpesviruses, was isolated from peripheral blood mononuclear cells of patients with lymphoproliferative disease [104]. Almost all children (95%) older than 2 years of age are currently seropositive for HHV-6A/B. The primary infection by HHV-6B sometimes causes *exanthem subitum* (roseola infantum), which is a febrile rash illness [105]. HHV-7, which also belongs to the same family of herpesviruses, was isolated in 1990 from the $CD4^+$ lymphocytes of a healthy person [106]. HHV-6 exists in variants A and B, and is a T-cell tropic virus that has been identified in various human tumors [107]. Patients with Burkitt's, African and EBV-negative B-cell lymphoma have been found to be seropositive for HHV-6, and especially HHV-6B [108–110]. In addition, variant 6A of the virus has been shown to contain a transformation suppressor gene, called *ts* [111]. Cell lines that transiently expressed the *ts* gene showed a significant resistance to the transformation activity of Ras. In the *ts*-expressing cells the transformation induced by Ras was reduced by ~98% when compared to the cells that do not express *ts*. Furthermore, the *ras* gene promoter expression was reduced by ~50%, indicating that even a small decrease of *ras* expression plays a very significant role in its ability to transform. *In vitro* studies have demonstrated that the viral *ts* gene can suppress the transcription from the HIV-1 long terminal repeat (LTR) promoter, as well as *ras* transformation at the promoter level. The transformation suppression that takes place in *ts*-expressing HHV-6 infected cells is attributed to the binding site of *ts* which is common between the LTR promoter and *ras* [112]. Unfortunately, there are insufficient scientific data available today to verify whether HHV-6 and -7 are able to implicate the Ras signaling pathway to induce increased permissiveness or altered cellular transformation. However, HHV-6 has been detected in patients that develop *ras*-associated types of cancers such as bladder cancer [113] or non-melanoma skin tumors [114], raising questions about the role the virus might play in these patients.

4. Gamma-herpesviruses

The gamma-herpesviruses are the third subfamily of herpesviruses, including the Epstein–Barr virus and Kaposi's Sarcoma-Associated Herpesvirus. EBV and KSHV are characterized as lymphotropic viruses, since they both have a cellular tropism for lymphocytes. Unlike other human herpesviruses, the latent phase of EBV and KSHV can be studied *in vitro*, providing an experimental system that is unique among human herpesviruses. Latency of gamma-herpesviruses is established after initial infection of the host cell and transport of the capsid to the nucleus. The viral genome is then able to replicate as an episome, utilizing the replication mechanisms of the host cell. A unique characteristic of gamma-herpesviruses is their ability to induce abnormal lymphocyte proliferation and tumorigenesis. The balance between lytic and latent infection is one of the most critical questions in modern virology research. Although the exact mechanisms of gamma-herpesviral reactivation need further study, it is believed that lytic replication plays a crucial role in tumorigenesis [115]. Especially for KSHV, low levels of periodical reactivation may lead to disease development in the host and subsequent viral transmission to uninfected cells. Viral reactivation in the host cell could also be explained by the cell's microenvironment. For example, activation of certain signaling pathways and the release of cytokines from the host cell as a response to inflammation may provide a suitable environment for the proliferation of infected cells [116].

4.1. Epstein–Barr virus (EBV)

EBV is able to infect a variety of cell types in the human body, depending on the circumstances. Cells infected by EBV include NK-, T-, smooth muscle and possibly follicular dendritic cells [117]. Several human types of cancer such as Nasopharyngeal Carcinoma (NPC), Burkitt's lymphoma (BL) and Hodgkin's disease (HD) have also been attributed to EBV infection [118].

However, the virus preferentially infects and resides latently in quiescent B memory cells [119]. Of note, however, is the mechanism that the virus uses to evade host-cell immunity [120]. Once in the latent phase, the virus is able to express several gene expression motifs, depending on the location and differentiation state of the host B cell [121]. In order to successfully remain in a latent state for an extended period of time, EBV expresses a small subset of viral latent genes that encode six Epstein–Barr nuclear antigens (EBNA1, 2, 3A, 3B, 3C, 4, 5 and 6), three membrane proteins (LMP1, LMP2A and LMP2B), BART, as well as two small nuclear RNA molecules (EBER 1 and 2) [122]. The abovementioned genes are expressed in four distinct patterns: type 0, 1, 2 and 3 [117,123,124]. During type 0 latency, EBV may not express any genes or may only express EBNA1 or LMP2. In type 1, latency involves the expression of EBNA1 and EBER1 and 2 in Burkitt's lymphoma (BL) patients. In type 2 latency which is found in Nasopharyngeal Carcinoma (NPC) and Hodgkin's lymphoma (HL) patients, EBNA1, LMP1 and 2, EBERs and BARTs are expressed. Finally, in the type 3 latency expression pattern, BARTs and EBERs are expressed. Notably, of all the genes encoded by EBV during latency only the LMP2A membrane protein induces the activation of the Ras protein *in vivo*. The activated Ras protein induces the PI3K/Akt cascade, but not the Raf/MEK/ERK pathway, to provide B-cell survival and resistance to apoptosis (Fig. 1) [125,126].

EBV is also able to infect epithelial cells and affect cellular transformation. To accomplish this, the virus expresses the integral viral membrane protein LMP1. LMP1 has been found to be directly associated with the activation of the MAPK cellular pathway in order to activate the transcription factor AP-1 and regulate cell cycle [127]. Activation of a Ras-MAPK-dependent pathway by Epstein–Barr virus latent membrane protein 1 causes prolonged ERK activation [128] which is essential for cellular transformation [129]. Furthermore, this pathway has been shown to contribute to the oncogenic nature of LMP1 through its ability to promote cell motility and to enhance the invasive properties of epithelial cells [130]. Therefore, LMP1 may act as a constitutively active mechanism that ensures the survival of EBV-infected B cells as well as their latency state, by interacting with the Ras pathway. LMP2A provides signals of survival and maturation for B cells, by inducing signaling patterns similar to the ones activated by latent B cells. LMP1 in particular has been shown to bind and interact with a vast number of transcription factors (AP-1), tumor necrosis factor receptors (TRAFs), as well as intracellular signaling molecules (p38, MAPKs, JNK), among others [131]. The increased binding affinity of LMP1 to host cell signaling cascades, such as the ras pathway, originates from the increased homology that the viral protein shares with the host-cell proteins.

4.2. Kaposi's sarcoma-associated Herpes Virus (KSHV)

KSHV is classified as an oncogenic virus which belongs to the gamma-herpesvirus family. DNA sequences suggesting the presence of a new virus were first described in 1994 [132]. KSHV is associated primarily with Kaposi's sarcoma [131,132], but is also known to cause Primary Effusion Lymphoma (PEL) [133] and the multicentric variant of Castleman Disease (MCD) [134]. During infection, the virus employs the integrin $\alpha 3/\beta 1$ subunit host-cell receptor in order to

gain entry into the cell [135]. Once inside the nucleus, the virus expresses a cascade of proteins that interact directly with cellular signaling pathway components. In more detail, KSHV is known to interact with the cytoplasmic, membrane-bound oncogenic protein Ras [131,136]. The activation of Ras results in the activation of a series of downstream components of both the PI3K and MAPK pathways as indicated in Fig. 1. The two pathways are activated by Ras and are responsible for cell growth, cell proliferation and programmed cell death as previously described in this review. In particular, the K8 viral protein, which is expressed during lytic infection, interacts with the extracellular signal-regulated kinase (ERK). In the presence of an ERK inhibitor (UO126) the expression of K8 was inhibited in a dose-dependent manner [137]. Interestingly, the latent KSHV infection of peripheral blood cells can shift towards lytic infection by chemical inducers such as 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) [138]. TPA induces lytic infection by activating the replication and transcriptional activation (RTA) protein, which is encoded by ORF50 [139,140]. RTA controls the virus reactivation process by directly or indirectly activating ORF59, vIL-6 and the K8 viral proteins. In addition, the RTA protein expression is induced by transcription factor AP-1, which is a downstream target of the three MAPK pathways mentioned above and is involved in cellular mechanisms of cell growth, cell transformation and angiogenesis. Hence, the expression of viral RTA protein is an indication of active viral replication and is directly regulated by MEK/ERK, JNK and the p38 MAPK pathways [141]. KSHV, as with all herpesviruses, is able to maintain a life-long infection by remaining latent in the host. KSHV then reactivates periodically by regulating cellular pathways such as MAPK and PI3K [138]. Activation of the p38/MAPK as well as the JNK signaling pathways is required for active KSHV infection to take place. Inhibition of these pathways produced a dose-dependent regulation of KSHV infection at the viral entry stage [142]. Additionally, the overexpression of Raf, a ras effector molecule and member of the Ras/Raf/MEK/ERK pathway, leads to increased KSHV infectivity, an event that takes place after attachment of the virus to the cell membrane [143]. In addition, KSHV latency involves the expression of the latent nuclear antigen 1 (LNA-1). Extensive research on the function of LNA-1 revealed that it acts synergistically with the H-*ras* protein to induce cellular proliferation and transformation [144].

5. Discussion

A significant amount of effort has been invested by numerous research groups in order to clarify the function of complex signaling cascades. The Ras/Raf/MEK/ERK pathway was initially considered to be a signal mediator, initiating outside the cell membrane and concluding in the cell nucleus. However, signaling pathways are implicated in a vast number of cellular functions, which in many cases contradict each other. In modern day research, it is known that information is transduced not only from the receptor to the nucleus, but also between cascades. Since a large number of molecules communicate with each other in the cytoplasm, the signal mediators serve as a “pyramid” of information. The key components of each pathway are located at the top of the “pyramid”. As the “pyramid” opens, various signal mediators, such as the MAPK kinases start to interact with molecules from different pathways to produce an enormous array of responses and signals.

It is known that most of the existing infectious agents predate the human race. Viruses in particular, have the capacity to evolve so rapidly that modern science struggles to sustain the pace. The frequent mutations that take place in herpesviral genomes produce novel capsid recognition sites specific to host-cell surface receptors, and ultimately aim to increase permissiveness to the host cell. Due to their prevalence in the worldwide population, herpesviruses have evolved to a point that they are able to manipulate host-cell mechanisms to achieve enhanced permissiveness and ensure latent infection. In their effort to replicate *in vivo*, herpesviruses alter the mechanisms of cell division, proliferation and cell death, including the Ras signaling pathway (Fig. 1).

The previous statement, however, does not fully apply to alpha-herpesviruses since there is no concrete evidence associating HSV-1/-2 infection to the altered proliferation of the host cell. Although several HSV proteins appear to employ components of the Ras signaling pathway, there are still a large number of viral proteins that have not been studied. Research on VZV appears to be even more obscure, probably due to the minimal scientific interest in the transformation properties of the virus.

Beta-herpesviruses interact with the cellular cyclin proteins in order to induce cell cycle arrest during infection. Once beta-herpesviruses are able to replicate within the cell, they manipulate the cell division process to increase the viral progeny. It is also widely known that beta-herpesviruses are able to block apoptosis, a process that would eventually result in their extinction. Although none of the beta-herpesviruses has been implicated in malignancy, their impact on the normal cell cycle and apoptosis raises significant questions on how they are able to modulate the cellular environment to their advantage.

Gamma-herpesviruses, on the other hand, directly implicate host-cell signaling processes in such a way that the physiological function of the cell is derailed, resulting in the abnormal transformation of the infected tissue. Furthermore, gamma-herpesviruses are able to both mimic ligands specific for receptors such as EGFR and employ such receptors in order to gain entry. The members of the MAPK family of kinases (MEKK, MAPK and ERK) are also targeted by gamma-herpesviruses. The Epstein–Barr virus, in particular, employs both the MEKK kinase and PI3K to increase its virulence. The Human Herpesvirus 8 is unique among herpesviruses due to its direct association with the Kaposi sarcoma and due to the number of cellular proteins it regulates. KSHV is able to interact with two molecules of the Ras pathway, Raf and Erk, while it indirectly induces the expression of PI3K and p38.

In summary, KSHV and EBV are the herpesviruses most involved in the Ras signaling pathway. KSHV employs the ras pathway by activating ras, PI3K, p38 and ERK1/2, whereas EBV activates MEK1/2 and PI3K kinases in order to promote cell proliferation and cell survival respectively. Additionally, HSV-1 and HSV-2 induce the expression of both *ras* and *raf* oncogenes, in order to promote prolonged proliferation of the host cell and establish a latent infection. Finally, HCMV, VZV and HHV6/7 are the less studied herpesviruses as far as the Ras signaling pathway is concerned. However, the discovery of the tumor-suppression activity of the HHV-6 *ts* gene in *ras*-overexpressing tumors is of great importance and its role should be investigated further, if we are to target HHV6 infection with gene therapy strategies. HCMV infection affects the pathway upstream of ras, at the tyrosine kinase receptor level, leaving many unanswered questions as to which specific molecules of the Ras pathway are induced and/or inhibited. Furthermore, VZV establishes a two-fold increase in p38/MAPK phosphorylation levels, proving that the pathway is susceptible to manipulation. What remains to be determined is whether the expression of VZV transcripts is associated with activation of upstream molecules such as Ras and Raf.

Due to the highly intrusive nature of herpesviruses, the host cell is rendered almost incapable of preventing the invasion. In addition, herpesviral genomes stay well hidden as episomes inside the host, opportunistically reactivating to cause severe inflammation and damage to the host. Consequently, the host cells are fated to become the “puppet” in the “hands” of the herpesvirus. Therefore, viral-mediated oncogenesis research needs to combine the research fields of Oncology and Virology. It is this unavoidable convergence of expertise that will elucidate the role of herpesviruses in cancer and ultimately produce a host-specific drug to combat cancer.

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