Gene Ther Mol Biol Vol 9, 51-60, 2005

Raf/MEK/ERK signaling: Implications in the infection and pathogenesis of viruses associated with AIDS

Review Article

Audy G. Whitman, Ossie F. Dyson, Patrick W. Ford, Shaw M. Akula*

Department of Microbiology & Immunology, Brody School of Medicine at East Carolina University, Greenville, NC 27834

***Correspondence**: Shaw M. Akula, Department of Microbiology & Immunology, Brody School of Medicine, East Carolina University, Greenville, North Carolina, USA 27834; Tel: (252) 744-2702; Fax: (252) 744-3104; E-mail: akulas@mail.ecu.edu

Key words: KSHV, HHV-8, AIDS, Raf

Abbreviations: acquired immunodeficiency syndrome, (AIDS); basic fibroblast growth factor, (bFGF); Epstein-Barr virus, (EBV); growth factor, (GF); heparin binding-epidermal growth factor, (HB-EGF); hepatitis B virus, (HBV); hepatitis C virus, (HCV); human cytomegalovirus, (HCMV); human herpesvirus-6, (HHV-6); human herpesvirus-8, (HHV-8); human immunodeficiency virus, (HIV); human papillomavirus, (HPV); human T-cell lymphotropic virus, (HTLV); inflammatory cytokines, (IC); interferons, (IFN); interleukins, (IL); Kaposi's sarcoma-associated herpesvirus, (KSHV); monocyte chemoattractant protein-1, (MCP-1); placental growth factor, (PLGF), granulocyte-macrophage colony-stimulating factor, (GM-CSF), Granulocyte colony-stimulating factor, (G-CSF); tumor growth factor, (TGF); tumor necrosis factor, (TNF); vascular endothelial growth factor, (VEGF)

Received: 7 March 2005; Accepted: 15 March 2005; electronically published: April 2005

Summary

Acquired immunodeficiency syndrome (AIDS) is characterized by failure of the immune system that is overwhelmed by an organized and well orchestrated invasion by various opportunistic pathogens. Cancers associated with AIDS are predominantly caused by viruses. Oncoproteins are directly involved in the initiation of neoplastic transformation. Recently, the presence of activating mutations in Raf has been described in a variety of cancers. Raf expression enhances Kaposi's sarcoma-associated herpesvirus (KSHV) infection of cells. KSHV is an etiology for Kaposi's sarcoma (KS); a condition commonly associated with AIDS. In addition, Raf associated signaling also regulates expression of various growth factors (GFs)/inflammatory cytokines (ICs). Since, AIDS is a condition that is regulated by aberrant GF/IC expression, we analyzed a possible role for Raf expression in the infection and pathogenesis of AIDS associated viruses in this review. This review also attempts to rationalize on why Raf associated signaling could well be a novel target to treat disease conditions due to viruses associated with AIDS.

I. Introduction

Acquired immunodeficiency syndrome (AIDS) is one of the most destructive pandemics that is getting worse with the progression of time (UNAIDS report for 2003). Human immunodeficiency virus (HIV) infection results in the progressive deterioration of the immune system resulting in AIDS. AIDS is characterized by an immunodeficient condition, which helps to facilitate opportunistic infections by bacteria (*Mycobacterium tuberculosis, Streptococcus pneumoniae, Salmonella enteritidis*), fungi (*Cryptococcus neoformans*), parasites (protozoan parasites of the genus *Plasmodium*), and viruses [human papillomavirus (HPV), human T-cell lymphotropic virus (HTLV), human polyomavirus (BK and JC virus), hepatitis B virus (HBV), hepatitis C virus (HCV), human herpesvirus-6 (HHV-6), human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), and Kaposi's sarcoma-associated herpesvirus (KSHV)] (Holmes et al, 2003; Whitman et al, 2004). Of these, most cancers seen in AIDS setting are related to oncogenic viruses like those listed above (Aoki and Tosato, 2004). Pathogenesis mediated by the viruses associated with AIDS is reviewed in our earlier work (Whitman et al, 2004).

The first signaling pathway to be delineated was the Raf/MEK/ERK cascade. The extracellular signal-regulated kinase (ERK) constitutes a highly conserved signaling module that is activated in mammalian cells via stimulation of receptor tyrosine kinases, G-protein coupled receptors and integrins (Widmann et al, 1999). This signal cascade is broadly referred to as the mitogen activated protein (MAP) kinase (MAPK) module: Raf/MEK/ERK.

This pathway regulates a variety of cellular processes, including embryogenesis, proliferation, differentiation and apoptosis, among others each of which plays a significant role in tumorigenesis (Hilger et al, 2002; Hindley and Kolch, 2002). However, deregulated Raf expression and activation as well as mutated forms of the Raf oncogenes have been observed in a wide variety of cancers (Davies et al, 2002; Rajagopalan et al, 2002). Over the last couple of years, our lab has been focusing on oncoprotein Raf and its role in KSHV infection of target cells and pathogenesis. There are several oncoproteins identified thus far: BCR-ABL, Bcl-2, Kit, Myc, Neu, Ras, Raf, Src, and others (Peles and Yarden, 1993; Grandori and Eisenman, 1997; Sawyers, 1997; Klasa et al, 2002; Kitamura and Hirotab, 2004; Martin, 2004; Wellbrock et al, 2004a). Initially, we decided to analyze the effect of Raf on KSHV infection of target cells due to the following reasons: (1) Raf has been identified as an oncogene playing a critical role in human cancers (Davies et al, 2002; Wellbrock et al, 2004b). B-Raf somatic missence mutations have been detected in malignant melanomas (66%) and colon cancers (15%) and at lower frequencies in a variety of human cancers (Davies et al, 2002). Raf proteins play a key role in the conserved Ras/Raf/MEK/ERK pathway, acting to relay signals from activated Ras proteins via MEK1/2 to ERK1/2, the key effectors of this pathway (Sebolt-Leopold, 2000), (2) a broad array of solid tumors are known to express constitutively phosphorylated ERK1/2 which is a downstream event of Ras/Raf signaling (Mercer and Pritchard, 2003), and (3) constitutive activation of the components (Ras/Raf) of the MAPK signaling pathway has been associated with a variety of tumors including AIDS-KS (Faris et al, 1996). It is almost two years since we started to work on Raf; very interesting findings have been identified and never ceases to amaze us. The purpose of this review is to summarize recent findings on the effects of Raf associated signaling in the infection and pathogenesis of viruses associated with AIDS. In addition, we also rationalize on why targeting Raf associated signaling may serve the purpose in controlling cancer induced by some of the viruses associated with AIDS.

II. Effect of Raf/MEK/ERK on virus infection and pathogenesis

A detailed description on Raf and its involvement in tumor formation was discussed previously (Whitman et al, 2004; Hamden et al, 2005). Viruses exploit the already available cellular signaling to their advantage; be it entry, infection, replication, or egress (Popik and Pitha, 2000; Hasler and Zouali, 2003; Hayward, 2004). Raf, and its associated signaling components Ras, MEK, and ERK, have been shown to aid in the infection and pathogenesis of viruses, including those associated with AIDS. The effects of Raf/MEK/ERK signaling on viruses associated with AIDS are described below:

A. HIV

HIV-1 infection predisposes to the development of specific types of cancer caused predominantly by EBV, KSHV, and HPV. HIV has a direct and/or indirect role to play in the development of cancers in AIDS patients by the above listed oncogenic viruses (Aoki and Tosato, 2004). Binding of HIV-1 to CD4 receptors is sufficient to stimulate the activation of Raf/MEK/ERK signaling in a Ras independent manner (Popik and Pitha, 1996; Popik et al, 1998). Binding of HIV-1 to CD4 receptors stimulate association of Lck with Raf-1 leading to the activation of the Raf associated signaling which eventually results in the expression of cytokine and chemokine genes. Interestingly, this process is independent of the Raf associated signaling induced by the stromal cell-derived factor 1 (SDF-1) binding CXCR4, which inhibits entry of T-cell-tropic HIV-1. In fact, SDF-1 binding to CXCR4 activates the Raf/MEK/ERK signaling but does not affect the CD4-mediated expression of cytokine and chemokine genes (Popik et al, 1998). Treatment of cells with MAPK stimulators, as well as coexpression of constitutively activated Ras, Raf, or MEK-enhanced HIV-1 infection of cells (Yang and Gabuzda, 1999). On the contrary, inhibition of the MAPK/ERK kinase pathways by dimethylamiloride lowered HIV infection of cells. The blocking of HIV-1 entry into target cells by dimethylamiloride indicates that HIV-1 uses macropinocytosis to enter endothelial cells (Liu et al, 2002). Recently, the same research group demonstrated that the MEK inhibitor, U0126 significantly inhibited the ERK1/2 phosphorylation initiated by HIV-1, gp120, or Tat in neonatal rat ventricular myocytes (NRVM) and coronary artery endothelial cells (CAEC) (Fiala et al, 2004). Other studies have shown target cells exposed to growth factors such as stromal cell-derived factor-1 (SDF-1) leads to an increased expression of the X4 strain of HIV-1 that is significantly inhibited by MEK inhibitors (Montes et al, 2000).

B. HBV and HCV

HBx protein of HBV has been reported to stimulate the Ras/Raf/MAPK cascade of signaling which may play a role in viral pathogenesis (Benn et al, 1996) while, the HCV core protein activates the Ras/Raf signaling pathway independent of the entire viral machinery (Shimotohno et al, 2002).

C. HCMV

HCMV is detected at a higher frequency in human tumor cells than it is in surrounding tissues. This is because, HCMV promotes cell survival by influencing tumor suppressor p53 and p73, and also by stimulation of the antiapoptotic Ras/Raf/MEK/ERK and PI-3K signaling pathways (Michaelis et al, 2004).

D. EBV

Activation of the Ras/Raf/MEK/ERK signaling pathway by EBV encoded latent membrane protein (LMP) is essential for transformation in fibroblasts (Roberts and Cooper, 1998).

E. KSHV

KSHV is also referred to as human herpesvirus-8 (HHV-8). KSHV is etiologically associated with all forms

of Kaposi's sarcoma (KS), multicentric Castleman disease (MCD), and primary effusion lymphoma (PEL) (Chang et al, 1994; Ganem, 1998). Oncoprotein Raf enhances KSHV infection of target cells (Akula et al, 2004). In two separate studies, we demonstrated the ability of Raf/MEK/ERK signaling to induce vascular endothelial growth factor (VEGF) expression in cells (Ford et al, 2004; Hamden et al, 2004). Recently, we demonstrated that the Raf induced VEGF plays a critical role in mediating angiogenesis (Akula et al, 2005); a feature of all KSHV mediated pathogenesis (Gallo, 1998a; Ganem, 1998).

There is a dearth of knowledge on the effect of Raf associated signaling on HTLV-1, HPV, and HHV-6. This does not rule out a possible role for Raf in these virus infections. It should be remembered that the Raf associated signaling is not always essential for virus infection. Simian virus 40 (SV40) T antigen stimulates Raf signaling to transform both insect and mammalian cells (Raptis et al, 1997; Grammatikakis et al, 2001); however, SV40 binding to target cells does not activate Raf/MEK/ERK. Instead, binding of SV40 to target cells activate a tyrosine kinase and Ca^{2+}-independent isoform of protein kinase C (PKC). SV40 infectious entry into cells was specifically and reversibly blocked by genistein (Dangoria et al, 1996). On the same lines, there are also instances where disruption of the Raf/MEK/ERK pathway has been demonstrated to aid in the infection of some viruses. Inhibition of MEK has been shown to up-regulate coxsackievirus and adenovirus receptors (CAR) expression in highly malignant cell lines. This specific disruption of the Raf/MEK/ERK pathway and increased expression of receptors enhances virus entry into the target cell (Anders et al, 2003).

III. Effect of growth factors (GFs)/inflammatory cytokines (ICs) on viral disease conditions

Growth Factors are serum proteins that regulate several cellular functions when they bind to specific cell-surface receptors. Growth factors mediate both the development as well as adaptive and pathological changes in the system (Waltenberger, 2005). Inflammatory Cytokines are chemical communicators between cells that regulate inflammatory responses and immune functions (Alfano and Poli, 2005). Pathogenesis is the cellular events and reactions that occur in the development of disease. Recent findings have suggested that upon infection, GFs and ICs are released by infected cells that promote infection of surrounding cells (Ganem, 1998; Aoki et al, 2000; Ensoli et al, 2000, 2001; Hayward, 2003; Hamden et al, 2005). Some examples of how GFs/ICs influence infection and pathogenesis of viruses associated with AIDS is listed below:

A. HIV

The hallmark of HIV-1 infection is a progressive dysfunction of the immune system, rendering the host vulnerable to a variety of opportunistic infections. Chronic HIV-1 infection leads to a virus-specific CD4(+) T-cell depletion accompanied by impaired antiviral cytotoxicity of $CD8^+$ T cells. Dysregulation of GFs/ICs is considered to be one of the factors that contribute to such an aberrant cell-mediated immunity (Zhang et al, 2003; Granelli-Piperno et al, 2004; Lee et al, 2004; Shapshak et al, 2004). Several GFs/ICS play a role in the HIV-1 initiated dysfunction of immune system. Some examples are, (1) studies have demonstrated that the excessive secretion of interleukin-18 (IL-18) may promote HIV-1 replication and thus the disease progression (Wiercinska-Drapalo et al, 2004), and (2) recent studies have shown an elevated circulating levels of interleukin-7 (IL-7) but not interleukin-15 (IL-15), transforming growth factor-β (TGF-β), and insulin-like growth factor-1 (IGF-1) during primary HIV-1 infection (Boulassel et al, 2004). Of these, IL-7 efficiently stimulates HIV-1 replication from $CD8^+$ T cell/monocyte–depleted PBMCs when compared to IL-2 (Wang et al, 2005b). They also demonstrated that IL-7 showed a positive trend for inducing proviral reactivation from resting $CD4^+$ T lymphocytes from HIV-1–infected patients on suppressive HAART.

B. HPV

ICs have been demonstrated to be released in response to HPV infection of the uterine cervix (Azar et al, 2004). Based on their results, they concluded that the detection of IL-10 and tumor necrosis factor-α (TNF-α) in cervical secretions may be a useful indicator of the stage of HPV induced cervical lesions. Interleukin-10 is one of the cytokines that is commonly elevated in cervical dysplasias or carcinomas as well as in the cervix of HIV-positive individuals. Interestingly, IL-10 also seems to enhance persistence and progression of HPV related disease conditions (Arany et al, 2002).

C. HTLV-1

Angiogenesis is a determining factor for the growth of both solid and hematologic tumors. HTLV-1 transformed cells secrete high levels of VEGF and basic fibroblast growth factor (bFGF); both of which mediate angiogenesis (Merhi et al, 2001). In another study it was demonstrated that the HTLV-1 infected cells produced high concentrations of interferon gamma (INF-γ) which was critical for migration of such cells through the peripheral tissues and thus contribute directly to the inflammation and disease condition (Hanon et al, 2001).

D. Human polyomavirus

JC virus produces its pathogenesis under immunosuppressive states such as AIDS. During the pathogenesis of AIDS, HIV-infected microglia secrete cytokines including interleukin-1 (IL-1) and TNF-α, which affect neuronal cells resulting in dysfunction of the central nervous system. Studies demonstrated the involvement of IL-1β mediated up-regulation of the JC virus early promoter (Kim et al, 2004). In another study, it was demonstrated that the HIV Tat protein-induced TGF-β1 augmented transcription of JCV promoter in glial cells (Enam et al, 2004).

E. HBV and HCV

GFs/ICs coordinate physiologic and pathologic processes going on in the liver and in pathogenesis mediated by HBV and HCV. A few examples of GF/IC mediated pathology are, (1) HBV encoded HBx may play a critical role in the hypoxia-induced angiogenesis through transcriptional activation of vascular endothelial growth factor (VEGF) during hepatocarcinogenesis (Lee et al, 2000), and (2) immunological studies demonstrate a vigorous cell-mediated immune response in HCV chronic liver disease and a deficient immune response in HBV chronic hepatitis (Missale et al, 1995).

F. HHV-6

HHV-6 is a potentially immunosuppressive agent that is suggested to act as a cofactor in the progression of HIV mediated disease conditions. Exposure of human macrophages to HHV-6 significantly lowered their ability to produce interleukin-12 (IL-12) (Smith et al, 2003). HHV-6 infection triggers monocytes to release monocyte chemoattractant protein-1 (MCP-1) and interleukin-10 (IL-10) (Arena et al, 2002). These findings implicate MCP-1 and IL-10 production to be closely related and that the marked level of MCP-1 is induced not only by the virus but also by virus-induced IL-10.

G. HCMV

EBV is the first human virus implicated in cancer. EBV utilizes its proteins in such a manner as to mimic several GFs, transcription factors, and antiapoptotic factors to control, regulate, and mediate its pathogenesis (Thompson and Kurzrock, 2004). BCRF1, BHRF1, and BARF1 are examples of important EBV encoded proteins that show sequence and functional homology to IL-10, BCL-2, and intracellular adhesion molecule 1, respectively (Thompson and Kurzrock, 2004). Latent membrane protein 1 (LMP-1) is one of the principle oncoproteins that is not only directly oncogenic but also can induce a broad range of GFs critical for the invasiveness of the cancers (Wakisaka and Pagano, 2003).

H. KSHV

KSHV pathogenesis is primarily dependent on the aberrant production of GFs/ICs (Gallo, 1998; Aoki and Tosato, 1999; Ensoli et al, 2001; Hayward, 2003). GFs/ICs play a major role in orchestrating several facets of KSHV pathogenesis such as activation of endothelial cells, recruitment of and activation of lymphocytes, mediating angiogenesis, vasculogenesis, and reactivation of latent infection (Gallo, 1998a). Some examples that best describe the role of GFs/ICs are, (1) IFN-γ and oncostatin-M has been demonstrated to induce lytic cycle of KSHV replication (Chang et al, 2000; Mercader et al, 2000), and (2) bFGF acts synergistically with VEGF and influences cell growth, migration, differentiation, and angiogenesis. It is said to play a key role along with VEGF in the progression of tumor (Ensoli et al, 2001). In addition, we determined that GFs like VEGF and heparin-binding epidermal growth factor (HB-EGF) can enhance KSHV infection of cells (Akula et al, 2004; Hamden et al, 2004). We have previously discussed in detail on the role for GFs/ICs in KSHV mediated pathogenesis (Hamden et al, 2005).

IV. Targeting Raf/MEK/ERK: a novel strategy to counter cancers mediated by AIDS associated viruses

We propose targeting Raf/MEK/ERK signaling to be an effective and novel strategy to treat cancer mediated by AIDS associated viruses due to the following reasons,

(1) The role for Raf associated signaling is not extensively studied in virus research when compared to tumor biology. However, after the advent of success with the use of Raf and other signaling inhibitors in treating tumor conditions, there have been several reports and suggestions about treating virus infections by manipulating the cellular signaling pathways. Understanding virus induced/utilized signals may lead to successful strategies for targeting specific signaling molecules to develop anti-viral therapies. Recent studies have demonstrated that the drugs targeting the MAPK and ErbB-signaling pathways represent a promising new class of antiviral agents to treat SARS and poxvirus infections, respectively (Mizutani et al, 2004; Fauci and Challberg, 2005; Yang et al, 2005).

(2) Raf associated signaling seems to mediate virus infection and/or pathogenesis (section I).

(3) An elevated expression of Raf/MEK/ERK signaling is common among solid tumors (Mercer and Pritchard, 2003). In a recently concluded study we also demonstrated that the hematopoietic cells derived from KSHV infected primary effusion lymphoma (PEL) expressed elevated levels of Raf/MEK/ERK activity when compared to uninfected B cells (Akula et al, 2005).

(4) Drugs targeting signaling pathways such as Raf/MEK/ERK may be apt because of the decrease in possibility of the development of mutations that bestow resistance (Ferrara, 2004).

(5) Finally and more importantly, Raf holds a pivotal position in the MAPK signaling pathway (Hamden et al, 2005). Research has identified the ability of several other key signaling molecules (Ras, JAK, PKA, Rap, Src family kinase, Rac, PI3K, PDK-1, Akt) to transduce signaling via Raf; and interestingly, MEK seems to be one of the well characterized substrate for Raf resulting in the regulation of a variety of GFs/ICs expression (Hamden et al, 2005; **Table 1**).

All the above mentioned factors make the Raf/MEK/ERK a therapeutic target to cure disease conditions associated with a variety of AIDS related viruses (Hamden et al, 2005). There are several inhibitors specific for the Raf/MEK/ERK signaling pathway being tested for their ability to prevent cancers. Some of the popular Raf/MEK/ERK inhibitors are,

(1) BAY 43-9006 (Onyx Pharmaceuticals): The compound binds to the Raf substrate-binding cleft with a great affinity and blocks the ATP binding site. BAY 43-9006 is in phase III clinical trails for the treatment of various cancers (Lowinger et al, 2002; Strumberg and Seeber, 2005).

Table 1. Raf/MEK/ERK signaling regulates expression of GFs/ICs

GF/IC	Mechanism of the Raf/MEK/ERK mediated GF/IC expression
bFGF	Cyclic AMP-induced redirection of bFGF signaling is mediated via the Raf/MEK/ERK signaling pathway in glial cells (Bayatti and Engele, 2001).
G-CSF	NS-398, a selective COX 2 inhibitor, lowers G-CSF production via MAPK signaling pathway in lung cancer cell lines OKa-C-1 and MI-4 (Nakata et al, 2003). MEK inhibitors PD98059 and U0126 also allowed a full rescue of G-CSF-induced neutrophilic differentiation (Jorda et al, 2003).
GM-CSF	GM-CSF expression is induced by a new cytokine, ML-1 (IL-17F) through the activation of the Raf-1/MEK/ERK signaling pathway (Kawaguchi et al, 2004). MEK inhibitors reduce ERK cascade activation and thus preferentially inhibit GM-CSF production. They concluded that the activation of Raf/MEK/ERK leads to the up-regulation of GM-CSF expression in cells (Matsubara et al, 2005).
HB-EGF	Activation of Raf-1:ER and a conditional oncogenic form of B-Raf, delta B-Raf:ER, results in rapid induction of HB-EGF mRNA and secretion of mature HB-EGF from cells (Akula et al, 2004; McCarthy et al, 1995).
IL-1β	Activation of the MAPK pathway of signaling is involved in the A beta-induced expression of IL-1β, IL-8, and MCP-1 (Giri et al, 2003).
IL-6	It has been shown that Raf-1 is required for hematopoietic growth-factor-induced proliferation of murine cell lines, one of them being IL-6. This was concluded using c-raf antisense oligonucleotides to block Raf-1 (Muszynski et al, 1995). Histamine activates Ca2+-dependent PKC isoforms that activates the Raf/MEK/ERK cascades that leads to up-regulation of IL-6, and IL-8 (Matsubara et al, 2005).
IL-8	The Raf/MEK/ERK pathway is a necessity for the IL-8 expression induced by Adenovirus type 7; and this occurs at the level of transcription (Alcorn et al, 2001). Others have also reported that the Raf/MEK/ERK pathway is crucial for the activation of IL-8 (Matsubara et al, 2005).
IL-10	IL-10 levels in monocyte supernatant were inhibited by MEK inhibitors, PD98059 and Ro 09-2210 (van der Bruggen et al, 1999). Other studies have documented that the down-regulation and/or inactivation of the MAPK pathway is correlated with an up-regulation in IL-10 expression; as of now the exact phenomenon is not deciphered (Man et al, 2005).
IL-12	Candida albicans inhibits IL-12 production by human monocytes by secreting an ERK44/42 MAPK-stimulating factor and thus can attenuate effective immune responses (Tang et al, 2004).
IFN-γ	The inhibition of the MEK/ERK pathway results in an enhanced, anti-proliferative effect of IFN-γ (Romerio and Zella, 2002).
IFN-γ	Raf kinase is a key mediator in T cell signaling crucial for the expression of IFN-γ in Th1 cells (Webber et al, 1997).
MCP-1	Glycated human serum albumin was shown to stimulate IL-8 and MCP-1 production which was found to be predominately mediated by the MAPK signaling pathway (Bian et al, 2001). Another study reported that the ERK1/2 activation in THP-1 monocytes is involved in the expression of various GFs/ICs, including MCP-1 (Giri et al, 2003).
PlGF	There is little evidence on the direct role of Raf in PlGF expression. However, it should be noted that PlGF is a member of the VEGF family, and there is an abundant amount of data indicating that Raf regulates VEGF expression (Akula et al, 2004; Hamden et al, 2004; Weinstein-Oppenheimer et al, 2002).
TNF-α	In DC2.4 dendritic cells Raf is phosphorylated and involved in the production of TNF-β and tyrosine phosphorylation of P13-K via ERK (Nakayama et al, 2003).
TNF-β	In Jurkat cells or peripheral blood T lymphocytes, the p21 Ras/Raf/MEK-E1 cascade plays a role in regulating the production of TNF-β (Li et al, 1999).
TGF-β1	MAPK inhibitor, such as PD98059, blocks the collaborative activities of RON and TGF-,1 in alpha-SMA expression induction, thus demonstrating a regulating role of the MAPK pathway in the expression of TGF-β1 (Wang et al, 2005).
VEGF	Overexpression of Raf in human foreskin fibroblasts induces expression of VEGF (Akula et al, 2004; Hamden et al, 2004). Other research has demonstrated that the disruption of the interaction between retinoblastoma tumor suppressor protein (Rb) and Raf-1 causes the inhibition of the VEGF-mediated capillary tubule formation (Dasgupta et al, 2004).

(2) Tyrphostin AG 879: The compound markedly inhibits the expression of the Raf-1 and HER-2 genes (Larsson, 2004).

(3) 17-AAG: An Hsp90 antagonist with a unique ability to inhibit multiple survival pathways, including Raf associated signaling (Rahmani et al, 2004). This is currently in phase I clinical trials (Neckers, 2002).

(4) ZM 336372: A protein kinase inhibitor that can specifically inhibit Raf kinase activity. It is still in preclinical stages of testing (Hill-Jackson et al, 1999).

(5) ISIS 5132: It is a 20-base anti-sense phosphorothioate oligodeoxyribonucleotide that specifically inhibits Raf-1 kinase activity. This has been found to be promising in phase II clinical trials (Gibbs, 2000).

(6) PD 184352 (CI-1040; Pfizer): It has been demonstrated to be well tolerated in phase I clinical trials (Allen et al, 2003). It inhibits phosphorylation of MEK1/2 by binding ATP and ERK1/2 binding sites on MEK1/2 (Lowinger et al, 2002).

(7) ARRY-142886 (AZD6244; AstraZeneca and Array BioPharma): It has been impressive in animal models for human cancers and is being proposed as one of the promising second generation MEK inhibitors. Interestingly, it is orally active, with a clean tolerability profile, and highly selective to MEK (Hamden et al, 2005).

V. Conclusion

At this point we are far from having completely understood Raf associated signaling and its influence on the cellular functions with respect to virus infection and pathogenesis. However, the role for Raf in tumor biology has been well documented. Raf signaling mediates tumor cell proliferation, cell migration, cell invasion, and angiogenesis; all of which are a hallmark of tumorigenesis (Bian et al, 2004; Wilhelm et al, 2004; Akula et al, 2005; Shin et al, 2005). Accordingly, Raf inhibitors have been used to treat various cancers that include breast cancer, lung adenomas, pancreatic cancer, thyroid cancer, and others (Kramer et al, 2004; Larsson, 2004; Sebolt-Leopold, 2004; Williams and Smallridge, 2004; Xiong, 2004). We are confident that the knowledge on the role of Raf/MEK/ERK signaling in virus mediated pathogenesis will grow substantially and that it can be used in the future to treat infection/pathogenesis mediated by viruses related to AIDS.

Acknowledgments

The work was supported in part by an Institutional Grant from American Cancer Society (IRG-97-149) to SMA. We thank Dr. Vishnu Chintalgattu and Balaji M. Akula for critically reading this manuscript. We also thank a past member of the lab, Khalief E. Hamden for his insightful discussion and sharing of ideas.

References

Akula SM, Ford PW, Whitman AG, Hamden KE, Bryan BA, Cook PP, and McCubrey JA (**2005**) B-Raf dependent expression of vascular endothelial growth factor-A in Kaposi's sarcoma-associated herpesvirus infected human B cells. **Blood** (In Press).

Akula SM, Ford, PW, Whitman AG, Hamden KE, Shelton JG, and McCubrey JA (**2004**) Raf promotes human herpesvirus-8 (HHV-8/KSHV) infection. **Oncogene** 23, 5227-5241.

Alcorn MJ, Booth JL, Coggeshall KM, and Metcalf JP (**2001**) Adenovirus type 7 induces interleukin-8 production via activation of extracellular regulated kinase ó. **J Virol** 75, 6450-6459.

Alfano M, and Poli G (**2005**) Role of cytokines and chemokines in the regulation of innate immunity and HIV infection. **Mol Immunol** 42, 161-182.

Allen LF, Sebolt-Leopold J, and Meyer MB (**2003**) CI-1040 (PD184352), a targeted signal transduction inhibitor of MEK (MAPKK). **Semin Oncol** 30, 105-116.

Anders M, Christian C, McMahon M, McCormick F, and Korn WM (**2003**) Inhibition of the Raf/MEK/ERK pathway up-regulates expression of the coxsackievirus and adenovirus receptor in cancer cells. **Cancer Res** 63, 2088-2095.

Aoki Y, and Tosato G (**1999**) Role of vascular endothelial growth factor/vascular permeability factor in the pathogenesis of Kaposi's sarcoma-associated herpesvirus-infected primary effusion lymphomas. **Blood** 94, 4247-4254.

Aoki Y, and Tosato G (**2004**) Neoplastic conditions in the context of HIV-1 infection. **Curr HIV Res** 2, 343-349.

Aoki Y, Yarchoan R, Braun J, Iwamoto A, and Tosato G (**2004**) Viral and cellular cytokines in AIDS-related malignant lymphomatous effusions. **Blood** 96, 1599-1601.

Arany I, Grattendick KG, and Tyring SK (**2002**) Interleukin-10 induces transcription of the early promoter of human papillomavirus type 16 (HPV16) through the 5'-segment of the upstream regulatory region (URR). **Antiviral Res** 55, 331-339.

Arena A, Stassi G, Speranza A, Iannello D, and Mastroeni, P (**2002**) Modulatory effect of HHV-6 on MCP-1 production by human monocytes. **New Microbiol** 25, 335-340.

Azar KK, Tani M, Yasuda H, Sakai A, Inoue M, and Sasagawa T (**2004**) Increased secretion patterns of interleukin-10 and tumor necrosis factor-alpha in cervical squamous intraepithelial lesions. **Hum Pathol** 35, 1376-1384.

Bayatti N, and Engele J (**2001**) Cyclic AMP modulates the response of central nervous system glia to fibroblast growth factor-2 by redirecting signalling pathways. **J Neurochem** 78, 972-980.

Benn, J., Su, F., Doria M, and Schneider RJ (**1996**) Hepatitis B virus HBx protein induces transcription factor AP-1 by activation of extracellular signal-regulated and c-Jun N-terminal mitogen-activated protein kinases. **J Virol** 70, 4978-4985.

Bian D, Su S, Mahanivong C, Cheng, RK, Han Q, Pan ZK, Sun P, and Huang S (**2004**) Lysophosphatidic Acid Stimulates Ovarian Cancer Cell Migration via a Ras-MEK Kinase 1 Pathway. **Cancer Res** 64, 4209-4217.

Bian ZM, Elner VM, Yoshida A, Kunkel SL, and Elner SG (**2001**) Signaling pathways for glycated human serum albumin-induced IL-8 and MCP-1 secretion in human RPE cells. **Invest Ophthalmol Vis Sci** 42, 1660-1668.

Boulassel MR, Young M, Routy JP, Sekaly RP, Tremblay C, and Rouleau D (**2004**) Circulating levels of IL-7 but not IL-15, IGF-1, and TGF-beta are elevated during primary HIV-1 infection. **HIV Clin Trials** 5, 357-359.

Chang J, Renne R, Dittmer D, and Ganem D (**2000**) Inflammatory cytokines and the reactivation of Kaposi's sarcoma-associated herpesvirus lytic replication. **Virology** 266, 17-25.

Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, and Moore PS (**1994**) Identification of herpesvirus-like

DNA sequences in AIDS-associated Kaposi's sarcoma. **Science** 266, 1865-1869.

Dangoria NS, Breau WC, Anderson HA, Cishek DM, and Norkin LC (**1996**) Extracellular simian virus 40 induces an ERK/MAP kinase-independent signalling pathway that activates primary response genes and promotes virus entry. **J Gen Virol** 77, 2173-2182.

Dasgupta P, Sun J, Wang S, Fusaro G, Betts V, Padmanabhan J, Sebti SM, and Chellappan SP (**2004**) Disruption of the Rb--Raf-1 interaction inhibits tumor growth and angiogenesis. **Mol Cell Biol** 24, 9527-9541.

Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, and Futreal PA (**2002**) Mutations of the BRAF gene in human cancer. **Nature** 417, 949-954.

Enam S, Sweet TM, Amini S, Khalili K, and Del Valle L (**2004**) Evidence for involvement of transforming growth factor beta1 signaling pathway in activation of JC virus in human immunodeficiency virus 1-associated progressive multifocal leukoencephalopathy. **Arch Pathol Lab Med** 128, 282-291.

Ensoli B, Sgadari C, Barillari G, Sirianni MC, Sturzl M, and Monini P (**2001**) Biology of Kaposi's sarcoma. **Eur J Cancer** 37, 1251-1269.

Ensoli B, Sturzl M, and Monini P (**2000**) Cytokine-mediated growth promotion of Kaposi's sarcoma and primary effusion lymphoma. **Semin Cancer Biol** 10, 367-381.

Faris M, Ensoli B, Stahl N, Yancopoulos G, Nguyen A, Wang S, and Nel AE (**1996**) Differential activation of the extracellular signal-regulated kinase, Jun kinase and Janus kinase-Stat pathways by oncostatin M and basic fibroblast growth factor in AIDS-derived Kaposi's sarcoma cells. **Aids** 10, 369-378.

Fauci AS, and Challberg MD (**2005**) Host-based antipoxvirus therapeutic strategies: turning the tables. **J Clin Invest** 115, 231-233.

Ferrara N (**2004**) Vascular endothelial growth factor as a target for anticancer therapy. **Oncologist** 9, 2-10.

Fiala M, Popik W, Qiao JH, Lossinsky AS, Alce T, Tran K, Yang W, Roos KP, and Arthos J (**2004**) HIV-1 induces cardiomyopathyby cardiomyocyte invasion and gp120, Tat, and cytokine apoptotic signaling. **Cardiovasc Toxicol** 4, 97-107.

Ford PW, Hamden KE, Whitman AG, McCubrey JA, and Akula SM (**2004**) Vascular Endothelial Growth Factor Augments Human Herpesvirus-8 (HHV-8/KSHV) Infection. **Cancer Biol Ther** 3, 876-881.

Gallo RC (**1998a**) The enigmas of Kaposi's sarcoma. **Science** 282, 1837-1839.

Gallo RC (**1998b**) HIV-1, HHV-8, and Kaposi's sarcoma. **J Hum Virol** 1, 185-186.

Ganem D (**1998**) Human herpesvirus 8 and its role in the genesis of Kaposi's sarcoma. **Curr Clin Top Infect Dis** 18, 237-251.

Gibbs JB (**2000**) Anticancer drug targets: growth factors and growth factor signaling. **J Clin Invest** 105, 9-13.

Giri RK, Selvaraj SK, and Kalra VK (**2003**) Amyloid peptide-induced cytokine and chemokine expression in THP-1 monocytes is blocked by small inhibitory RNA duplexes for early growth response-1 messenger RNA. **J Immunol** 170, 5281-5294.

Grammatikakis N, Jaronczyk K, Siganou A, Vultur A, Brownell HL, Benzaquen M, Rausch C, Lapointe R, Gjoerup O, Roberts TM, and Raptis L (**2001**) Simian virus 40 large tumor antigen modulates the Raf signaling pathway. **J Biol Chem** 276, 27840-27845.

Grandori C, and Eisenman RN (**1997**) Myc target genes. **Trends Biochem Sci** 22, 177-181.

Granelli-Piperno A, Golebiowska A, Trumpfheller C, Siegal FP, and Steinman RM (**2004**) HIV-1-infected monocyte-derived dendritic cells do not undergo maturation but can elicit IL-10 production and T cell regulation. **Proc Natl Acad Sci U S A** 101, 7669-7674.

Hall-Jackson CA, Eyers PA, Cohen P, Goedert M, Boyle FT, Hewitt N, Plant H, Hedge P (1999) Paradoxical activation of Raf by a novel Raf inhibitor. **Chem Biol** 6, 559-568.

Hamden KE, Ford PW, Whitman AG, Dyson OF, Cheng, SY, McCubrey JA, and Akula SM (**2004**) Raf-induced vascular endothelial growth factor augments Kaposi's sarcoma-associated herpesvirus infection. **J Virol** 78, 13381-13390.

Hamden KE, Whitman AG, Ford PW, Shelton JG, McCubrey, JA, and Akula SM (**2005**) Raf and VEGF: emerging therapeutic targets in Kaposi's sarcoma-associated herpesvirus infection and angiogenesis in hematopoietic and nonhematopoietic tumors. **Leukemia** 19, 18-26.

Hanon E, Goon P, Taylor GP, Hasegawa H, Tanaka Y, Weber JN, and Bangham CR (**2001**) High production of interferon gamma but not interleukin-2 by human T-lymphotropic virus type I-infected peripheral blood mononuclear cells. **Blood** 98, 721-726.

Hasler P, and Zouali M (**2003**) Subversion of B lymphocyte signaling by infectious agents. **Genes Immun** 4, 95-103.

Hayward GS (**2003**) Initiation of angiogenic Kaposi's sarcoma lesions. **Cancer Cell** 3, 1-3.

Hayward SD (**2004**) Viral interactions with the Notch pathway. **Semin Cancer Biol** 14, 387-396.

Helantera I, Loginov R, Koskinen P, Tornroth T, Gronhagen-Riska C, and Lautenschlager I (**2005**) Persistent cytomegalovirus infection is associated with increased expression of TGFβ1, PDGF-AA and ICAM-1 and arterial intimal thickening in kidney allografts. **Nephrol Dial Transplant** (In Press).

Hilger RA, Schelulen ME, and Strumberg D (**2002**) The Ras-Raf-MEK-ERK pathway in the treatment of cancer. **Onkologie** 25, 511-518.

Hindley A, and Kolch W (**2002**). Extracellular signal regulated kinase (ERK)/mitogen activated protein kinase (MAPK)-independent functions of Raf kinases. **J Cell Sci** 115, 1578-1581.

Holmes CB, Losina E, Walensky RP, Yazdanpanah Y, and Freedberg KA (**2003**) Review of human immunodeficiency virus type 1-related opportunistic infections in sub-Saharan Africa. **Clin Infect Dis** 36, 652-662.

Jorda MA, Lowenberg B, and Delwel R (**2003**) The peripheral cannabinoid receptor Cb2, a novel oncoprotein, induces a reversible block in neutrophilic differentiation. **Blood** 101, 1336-1343.

Kawaguchi M, Kokubu F, Odaka M, Watanabe S, Suzuki S, Ieki K, Matsukura S, Kurokawa M, Adachi M, and Huang SK (**2004**) Induction of granulocyte-macrophage colony-stimulating factor by a new cytokine, ML-1 (IL-17F), via Raf I-MEK-ERK pathway. **J Allergy Clin Immunol** 114, 444-450.

Kim SY, Choi EC, Woo Jo Y, Henson JW, and Kim HS (**2004**) Transcriptional activation of JC virus early promoter by phorbol ester and interleukin-1beta: critical role of nuclear factor-1. **Virology** 327, 60-69.

Kitamura Y, and Hirotab S (**2004**) Kit as a human oncogenic tyrosine kinase. **Cell Mol Life Sci** 61, 2924-2931.

Klasa RJ, Gillum AM, Klem RE, and Frankel SR (**2002**) Oblimersen Bcl-2 antisense: facilitating apoptosis in

anticancer treatment. **Antisense Nucleic Acid Drug Dev** 12, 193-213.

Kramer BW, Gotz R, and Rapp UR (**2004**) Use of mitogenic cascade blockers for treatment of C-Raf induced lung adenoma in vivo: CI-1040 strongly reduces growth and improves lung structure. **BMC Cancer** 4, 24.

Larsson LI (**2004**) Novel actions of tyrphostin AG 879: inhibition of RAF-1 and HER-2 expression combined with strong antitumoral effects on breast cancer cells. **Cell Mol Life Sci** 61, 2624-2631.

Lee S, French MA, and Price P (**2004**) IL-23 and IFN-gamma deficiency in immunodeficient HIV patients who achieved a long-term increase in CD4 T-cell counts on highly active antiretroviral therapy. **Aids** 18, 1337-1340.

Lee SW, Lee YM, Bae SK, Murakami S, Yun Y, and Kim KW (**2000**) Human hepatitis B virus X protein is a possible mediator of hypoxia-induced angiogenesis in hepatocarcinogenesis. **Biochem Biophys Res Commun** 268, 456-461.

Li YQ, Hii CS, Costabile M, Goh D, Der CJ, and Ferrante A (**1999**) Regulation of lymphotoxin production by the p21ras-raf-MEK-ERK cascade in PHA/PMA-stimulated Jurkat cells. **J Immunol** 162, 3316-3320.

Liu NQ, Lossinsky AS, Popik W, Li X, Gujuluva C, Kriederman, B, Roberts J, Pushkarsky T, Bukrinsky M, Witte M, Weinand M, and Fiala M (**2002**) Human immunodeficiency virus type 1 enters brain microvascular endothelia by macropinocytosis dependent on lipid rafts and the mitogen-activated protein kinase signaling pathway. **J Virol** 76, 6689-6700.

Lowinger TB, Riedl B, Dumas J, and Smith, RA (**2002**) Design and discovery of small molecules targeting raf-1 kinase. **Curr Pharm Des** 8, 2269-2278.

Man K, Ng KT, Lee TK, Lo, CM, Sun CK, Li XL, Zhao Y, Ho JW, and Fan ST (**2005**) FTY720 attenuates hepatic ischemia-reperfusion injury in normal and cirrhotic livers. **Am J Transplant** 5, 40-49.

Martin GS (**2004**) The road to Src. **Oncogene** 23, 7910-7917.

Matsubara M, Tamura T, Ohmori K, and Hasegawa K (**2005**) Histamine H1 receptor antagonist blocks histamine-induced proinflammatory cytokine production through inhibition of Ca2+-dependent protein kinase C, Raf/MEK/ERK and IKK/I kappa B/NF-kappa B signal cascades. **Biochem Pharmacol** 69, 433-449.

McCarthy SA, Samuels ML, Pritchard CA, Abraham JA, and McMahon M (**1995**) Rapid induction of heparin-binding epidermal growth factor/diphtheria toxin receptor expression by Raf and Ras oncogenes. **Genes Dev** 9, 1953-1964.

Mercader M, Taddeo B, Panella JR, Chandran B, Nickoloff BJ, and Foreman KE (**2000**) Induction of HHV-8 lytic cycle replication by inflammatory cytokines produced by HIV-1-infected T cells. **Am J Pathol** 156, 1961-1971.

Mercer KE, and Pritchard CA (**2003**) Raf proteins and cancer: B-Raf is identified as a mutational target. **Biochim Biophys Acta** 1653, 25-40.

Merhi RA, Guillaud L, Delouis C, and Cotinot C (**2001**) Establishment and characterization of immortalized ovine Sertoli cell lines. **In Vitro Cell Dev Biol Anim** 37, 581-588.

Michaelis M, Kotchetkov R, Vogel JU, Doerr HW, and Cinatl J., Jr (**2004**) Cytomegalovirus infection blocks apoptosis in cancer cells. **Cell Mol Life Sci** 61, 1307-1316.

Missale G, Ferrari C, and Fiaccadori F (**1995**) Cytokine mediators in acute inflammation and chronic course of viral hepatitis. **Ann Ital Med Int** 10, 14-18.

Mizutani T, Fukushi S, Saijo M, Kurane I, and Morikawa S (**2004**) Phosphorylation of p38 MAPK and its downstream targets in SARS coronavirus-infected cells. **Biochem Biophys Res Commun** 319, 1228-1234.

Montes M, Tagieva NE, Heveker N, Nahmias C, Baleux F, and Trautmann A (**2000**) SDF-1-induced activation of ERK enhances HIV-1 expression. **Eur Cytokine Netw** 11, 470-477.

Moon SK, Lee HY, Li JD, Nagura M, Kang SH, Chun YM, Linthicum FH, Ganz T, Andalibi A, and Lim DJ (**2002**) Activation of a Src-dependent Raf-MEK1/2-ERK signaling pathway is required for IL-1alpha-induced upregulation of beta-defensin 2 in human middle ear epithelial cells. **Biochim Biophys Acta** 1590, 41-51.

Muszynski KW, Ruscetti FW, Heidecker G, Rapp U, Troppmair J, Gooya JM, and Keller JR (**1995**) Raf-1 protein is required for growth factor-induced proliferation of hematopoietic cells. **J Exp Med** 181, 2189-2199.

Nakata H, Uemura Y, Kobayashi M, Harada R, and Taguchi H (**2003**) Cyclooxygenase-2 inhibitor NS-398 suppresses cell growth and constitutive production of granulocyte-colony stimulating factor and granulocyte macrophage-colony stimulating factor in lung cancer cells. **Cancer Sci** 94, 173-180.

Nakayama K, Ota Y, Okugawa S, Ise N, Kitazawa T, Tsukada K, Kawada M, Yanagimoto S, and Kimura S (**2003**) Raf1 plays a pivotal role in lipopolysaccharide-induced activation of dendritic cells. **Biochem Biophys Res Commun** 308, 353-360.

Neckers L (**2002**) Heat shock protein 90 is a rational molecular target in breast cancer. **Breast Dis** 15, 53-60.

Nordoy I, Rollag H, Lien E, Sindre H, Degre M, Aukrust P, Froland SS, and Muller F (**2003**) Cytomegalovirus infection induces production of human interleukin-10 in macrophages. **Eur J Clin Microbiol Infect Dis** 22, 737-741.

Peles E, and Yarden Y (**1993**) Neu and its ligands: from an oncogene to neural factors. **Bioessays** 15, 815-824.

Popik W, and Pitha PM (**1996**) Binding of human immunodeficiency virus type 1 to CD4 induces association of Lck and Raf-1 and activates Raf-1 by a Ras-independent pathway. **Mol Cell Biol** 16, 6532-6541.

Popik W, and Pitha PM (**2000**) Exploitation of cellular signaling by HIV-1: unwelcome guests with master keys that signal their entry. **Virology** 276, 1-6.

Popik W, Hesselgesser JE, Pitha PM (**1998**) Binding of human immunodeficiency virus type 1 to CD4 and CXCR4 receptors differentially regulates expression of inflammatory genes and activates the MEK/ERK signaling pathway. **J Virol** 72, 6406-6413.

Rahmani M, Reese E, Dai Y, Bauer C, Kramer LB, Huang M, Jove R, Dent P, and Grant S (2004) Co-treatment with SAHA and 17-AAG synergistically induces apoptosis in Bcr-Abl+ cells sensitive and resistant to STI-571 in association with down-regulation of Bcr-Abl, abrogation of STAT5 activity, and Bax conformational change. **Mol Pharmacol** (In Press).

Rajagopalan H, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B, and Velculescu VE (**2002**). Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. **Nature** 418, 934.

Raptis L, Brownell HL, Corbley MJ, Wood KW, Wang D, Haliotis T (**1997**) Cellular ras gene activity is required for full neoplastic transformation by the large tumor antigen of SV40. **Cell Growth Differ** 8, 891-901.

Roberts ML, and Cooper NR (**1998**) Activation of a ras-MAPK-dependent pathway by Epstein-Barr virus latent membrane protein 1 is essential for cellular transformation. **Virology** 240, 93-99.

Romerio F, and Zella D (**2002**) MEK and ERK inhibitors enhance the anti-proliferative effect of interferon-alpha2b. **Faseb J** 16, 1680-1682.

Rott D, Zhu J, Zhou YF, Burnett MS, Zalles-Ganley A, and Epstein SE (**2003**) IL-6 is produced by splenocytes derived

from CMV-infected mice in response to CMV antigens, and induces MCP-1 production by endothelial cells: a new mechanistic paradigm for infection-induced atherogenesis. **Atherosclerosis** 170, 223-228.

Sawyers CL (**1997**) Signal transduction pathways involved in BCR-ABL transformation. **Baillieres Clin Haematol** 10, 223-231.

Sebolt-Leopold JS (**2000**) Development of anticancer drugs targeting the MAP kinase pathway. **Oncogene** 19, 6594-6599.

Sebolt-Leopold JS (**2004**) MEK inhibitors: a therapeutic approach to targeting the Ras-MAP kinase pathway in tumors. **Curr Pharm Des** 10, 1907-1914.

Shapshak P, Duncan R, Minagar A, Rodriguez de la Vega P, Stewart RV, and Goodkin K (**2004**) Elevated expression of IFN-gamma in the HIV-1 infected brain. **Front Biosci** 9, 1073-1081.

Shimotohno K, Watashi K, Tsuchihara K, Fukuda K, Marusawa H, and Hijikata M (**2002**) Hepatitis C virus and its roles in cell proliferation. **J Gastroenterol** 37 Suppl 13, 50-54.

Shin I, Kim S, Song H, Kim HR, and Moon A (**2005**) H-ras-specific activation of Rac-MKK3/6-p38 pathway: Its critical role in invasion and migration of breast epithelial cells. **J Biol Chem** (In Press).

Smith A, Santoro F, Di Lullo G, Dagna L, Verani A, and Lusso P (**2003**) Selective suppression of IL-12 production by human herpesvirus 6. **Blood** 102, 2877-2884.

Strumberg D, and Seeber S (**2005**) Raf kinase inhibitors in oncology. **Onkologie** 28, 101-107.

Tang N, Liu L, Kang K, Mukherjee PK, Takahara M, Chen G, McCormick TS, Cooper KD, and Ghannoum M (**2004**) Inhibition of monocytic interleukin-12 production by Candida albicans via selective activation of ERK mitogen-activated protein kinase. **Infect Immun** 72, 2513-2520.

Thompson MP, and Kurzrock R (**2004**) Epstein-Barr virus and cancer. **Clin Cancer Res** 10, 803-821.

Van der Bruggen T, Nijenhuis S, van Raaij E, Verhoef J, and van Asbeck BS (**1999**) Lipopolysaccharide-induced tumor necrosis factor alpha production by human monocytes involves the raf-1/MEK1-MEK2/ERK1-ERK2 pathway. **Infect Immun** 67, 3824-3829.

Wakisaka N, and Pagano JS (**2003**) Epstein-Barr virus induces invasion and metastasis factors. **Anticancer Res** 23, 2133-2138.

Waltenberger J (**2005**) Growth factor signal transduction defects in the cardiovascular system. **Cardiovasc Res** 65, 574-580.

Wang D, Shen Q, Xu XM, Chen YQ, and Wang MH (**2005a**) Activation of the RON receptor tyrosine kinase attenuates transforming growth factor-beta1-induced apoptotic death and promotes phenotypic changes in mouse intestinal epithelial cells. **Carcinogenesis** 26, 27-36.

Wang FX, Xu Y, Sullivan J, Souder E, Argyris EG, Acheampong EA, Fisher J, Sierra M, Thomson MM, Najera R, Frank I, Kulkosky J, Pomerantz RJ, and Nunnari G (**2005b**) IL-7 is a potent and proviral strain-specific inducer of latent HIV-1 cellular reservoirs of infected individuals on virally suppressive HAART. **J Clin Invest** 115, 128-137.

Webber S, Zheng R, Kamal A, Withnall M, and Karlsson JA (**1997**) IFN-gamma production from human Th1 cells is controlled by Raf kinase. **Int Arch Allergy Immunol** 113, 275-278.

Weinstein-Oppenheimer C, Burrows C, Steelman L, and McCubrey J (**2002**) The effects of beta-estradiol on Raf activity, cell cycle progression and growth factor synthesis in the MCF-7 breast cancer cell line. **Cancer Biol Ther** 1, 256-262.

Wellbrock C, Karasarides M, and Marais R (**2004a**). The RAF proteins take centre stage. **Nat Rev Mol Cell Biol** 5, 875-885.

Wellbrock C, Ogilvie L, Hedley D, Karasarides M, Martin J, Niculescu-Duvaz D, Springer CJ, and Marais R (**2004b**) V599EB-RAF is an oncogene in melanocytes. **Cancer Res** 64, 2338-2342.

Whitman AG, Hamden KE, Ford PW, McCubrey, JA, and Akula SM (**2004**) Role for Raf in the entry of viruses associated with AIDS. **Int J Oncol** 25, 469-480.

Widmann C, Gibson S, Jarpe MB, and Johnson GL (1999) Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. **Physiol Rev** 79,143-180.

Wiercinska-Drapalo A, Jaroszewicz J, Flisiak R, and Prokopowicz D (**2004**) Plasma interleukin-18 is associated with viral load and disease progression in HIV-1-infected patients. **Microbes Infect** 6, 1273-1277.

Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, Chen C, Zhang X, Vincent P, McHugh M, Cao Y, Shujath J, Gawlak S, Eveleigh D, Rowley B, Liu L, Adnane L, Lynch M, Auclair D, Taylor I, Gedrich R, Voznesensky A, Riedl B, Post LE, Bollag G, and Trail PA (**2004**) BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. **Cancer Res** 64, 7099-7109.

Williams SF, and Smallridge RC (**2004**) Targeting the ERK pathway: novel therapeutics for thyroid cancer. **Curr Drug Targets Immune Endocr Metabol Disord** 4, 199-220.

Xiong HQ (**2004**) Molecular targeting therapy for pancreatic cancer. **Cancer Chemother Pharmacol** 54 Suppl 1, S69-77.

Yang H, Kim SK, Kim M, Reche PA, Morehead TJ, Damon IK, Welsh RM, and Reinherz EL (**2005**) Antiviral chemotherapy facilitates control of poxvirus infections through inhibition of cellular signal transduction. **J Clin Invest** 115, 379-387.

Yang X, and Gabuzda D (**1999**) Regulation of human immunodeficiency virus type 1 infectivity by the ERK mitogen-activated protein kinase signaling pathway. **J Virol** 73, 3460-3466.

Zhang D, Shankar P, Xu Z, Harnisch B, Chen G, Lange C, Lee SJ, Valdez H, Lederman MM, and Lieberman J (**2003**) Most antiviral CD8 T cells during chronic viral infection do not express high levels of perforin and are not directly cytotoxic. **Blood** 101, 226-235.

Shaw M. Akula