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Inhibition of host extracellular signal-regulated kinase (ERK) activation decreases new world alphavirus multiplication in infected cells

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ABSTRACT

New World alphaviruses belonging to the family Togaviridae are classified as emerging infectious agents and Category B select agents. Our study is focused on the role of the host extracellular signal-regulated kinase (ERK) in the infectious process of New World alphaviruses. Infection of human cells by Venezuelan equine encephalitis virus (VEEV) results in the activation of the ERK-signaling cascade. Inhibition of ERK1/2 by the small molecule inhibitor Ag-126 results in inhibition of viral multiplication. Ag-126-mediated inhibition of VEEV was due to potential effects on early and late stages of the infectious process. While expression of viral proteins was down-regulated in Ag-126 treated cells, we did not observe any influence of Ag-126 on the nuclear distribution of capsid. Finally, Ag-126 exerted a broad-spectrum inhibitory effect on New World alphavirus multiplication, thus indicating that the host kinase, ERK, is a broad-spectrum candidate for development of novel therapeutics against New World alphaviruses.

Introduction

The New World alphaviruses, Venezuelan equine encephalitis virus (VEEV), Eastern equine encephalitis virus (EEEV), and Western equine encephalitis virus (WEEV), can infect humans and potentially cause encephalitic disease (Weaver and Reisen, 2010). In 2013, Latin America recorded multiple confirmed cases of New World alphavirus infections with a total of 19 patients hospitalized for encephalitis. Among them, 3 patients died, 1 of whom had confirmed VEE (Carrera et al., 2013). Owing to their propensity to cause naturally-occurring disease, these alphaviruses have been classified as emerging infectious agents. The New World alphaviruses have the potential to be extremely infectious by the aerosol route and have therefore been explored previously as potential bioweapons (Zacks and Paessler, 2010). For this reason, VEEV and EEEV are classified by the Centers for Disease Control (CDC) as Category B select agents. There are currently no FDA-approved therapeutic candidates or vaccines for the protection of humans from New World alphavirus infections. The attenuated TC-83 strain of VEEV is used to vaccinate select at-risk personnel; however, TC-83 has concerns regarding safety and is considered to be a reactogenic vaccine (Barrett and Stanberry, 2009). Around 40% of all vaccinees have developed disease with some symptoms typical to that of natural VEE infection (Volkova et al., 2008). Therefore, there are ongoing efforts to establish a more effective and safer vaccine, some of which include vaccine candidates derived from the V3526 attenuated strain of VEEV (Fine et al., 2010; Martin et al., 2010; Paessler and Weaver, 2009; Sharma et al., 2011). FDA-approved antiviral therapies for RNA viruses, such as ribavirin, have been ineffective against VEEV and WEEV (Canonico et al., 1984), further highlighting the importance of new therapeutic approaches as medical countermeasures against New World alphaviruses.

Viruses rely on their host cell for the establishment of a productive infectious cycle. Viruses are obligate pathogens that are known to modulate and utilize many host events, including host signal-transduction mechanisms. A deeper understanding of the dynamics of the interactions between the host and the pathogen can help in the identification of novel targets for therapeutics. For example, many alphaviruses have developed the ability to interfere with the induction of the host cell antiviral response (Burke et al., 2009; Garmashova et al., 2007). Animal models have also revealed changes in gene expression in VEEV-infected mouse brains (Sharma et al., 2008). We have demonstrated that host kinases, such as

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glycogen synthase kinase-3β (GSK-3β) and the inhibitor of nuclear factor kappa-B kinase subunit beta (IKK-β), were modified upon VEEV infection (Kehn-Hall et al., 2012; Amaya et al., 2014). Targeting these kinases with small molecule inhibitors resulted in a decrease of viral replication. Such observations underscore the potential of host-based candidates as therapeutic targets for development of antivirals against New World alphaviruses. The advantages conferred by host-based therapeutics include a decreased potential for the development of resistant strains and an increased probability of broad-spectrum applicability to treat many viral indications. The concerns around host-based therapeutics include a low threshold for toxicity and hence, a requirement of a stringent analysis of inhibitor induced toxicity profiles in the host.

The host MAPK, extracellular signal-regulated kinase (ERK), responds to stress events including infection by directing multiple downstream events like inflammation and cell death (Hong et al., 2009; Hu et al., 2004; Xing et al., 2010). ERK exists as ERK1 and ERK2 (hereafter referred to collectively as ERK1/2), both of which have a central position in the MAPK cascade, downstream in RAS-RAF-MEK-ERK signal transduction (Roskoski, 2012a, 2012b). Briefly, RAF kinases act by phosphorylating and therefore activating MEK1 and MEK2 (collectively referred to as MEK1/2). MEK1/2 have dual specificity for ERK1/2, phosphorylating first at tyrosine and then threonine sites in the activation segments of ERK1/2, causing subsequent activation (Roskoski, 2012a, 2012b). Activated ERK1/2 act as protein-serine/threonine kinases, phosphorylating more than 150 cytosolic and nuclear substrates (Yoon and Seger, 2006; Shaul and Seger, 2007). ERK1/2 form an activated dimer and translocate to the nucleus, where they phosphorylate transcription factors regulating gene transcription (Chuderland and Seger, 2005; Parra et al., 2005).

Multiple viruses are known to activate the RAS–RAF–MEK–ERK signaling cascade in the host cell, and in many cases, this activation has been correlated with viral replication (Pleschka, 2008). Specifically, publications have implicated the ERK signaling pathway in the regulation of viral replication and gene expression for Coxsackievirus B3 (Luo et al., 2002), human cytomegalovirus (Boldogh et al., 1990; Johnson et al., 2001), Junin virus (Rodríguez et al., 2014), human immunodeficiency type 1 (Furler and Uittenbogaart, 2010; Jacqué et al., 1998), coronavirus (Cai et al., 2007), and influenza virus (Pleschka et al., 2001). Of particular relevance to our studies, it was reported that Borna disease virus, an RNA virus with high neurotropism, also manipulates the RAF/MEK/ERK signaling cascade in vitro and appears to be essential for viral spread (Hans et al., 2001; Planz et al., 2001). Here we demonstrate that VEEV infection of human astrocytoma cells results in the phosphorylation of multiple target proteins in the ERK signaling cascade. Using a small molecule inhibitor of ERK, Ag-126, we demonstrate that ERK1/2 phosphorylation plays an important role in VEEV multiplication in infected cells. We provide data which suggest that early and late events in the viral infectious cycle are susceptible to ERK1/2 inhibitors. We extend our studies to the virulent strains of VEEV, WEEV and EEEV, and demonstrate that ERK1/2 signaling is a broad-spectrum requirement for New World alphaviruses for the establishment of a productive infectious cycle.

Results

The RAF/MEK/ERK signaling cascade Is activated in VEEV-infected cells

Our previous studies have indicated that host kinases including IKK-β and GSK-3β were modulated in VEEV-infected cells and that inhibition of these kinases with small molecule inhibitors resulted in decreased viral multiplication (Amaya et al., 2014; Kehn-Hall et al., 2012). We reported in our IKK-β study that multiple components,

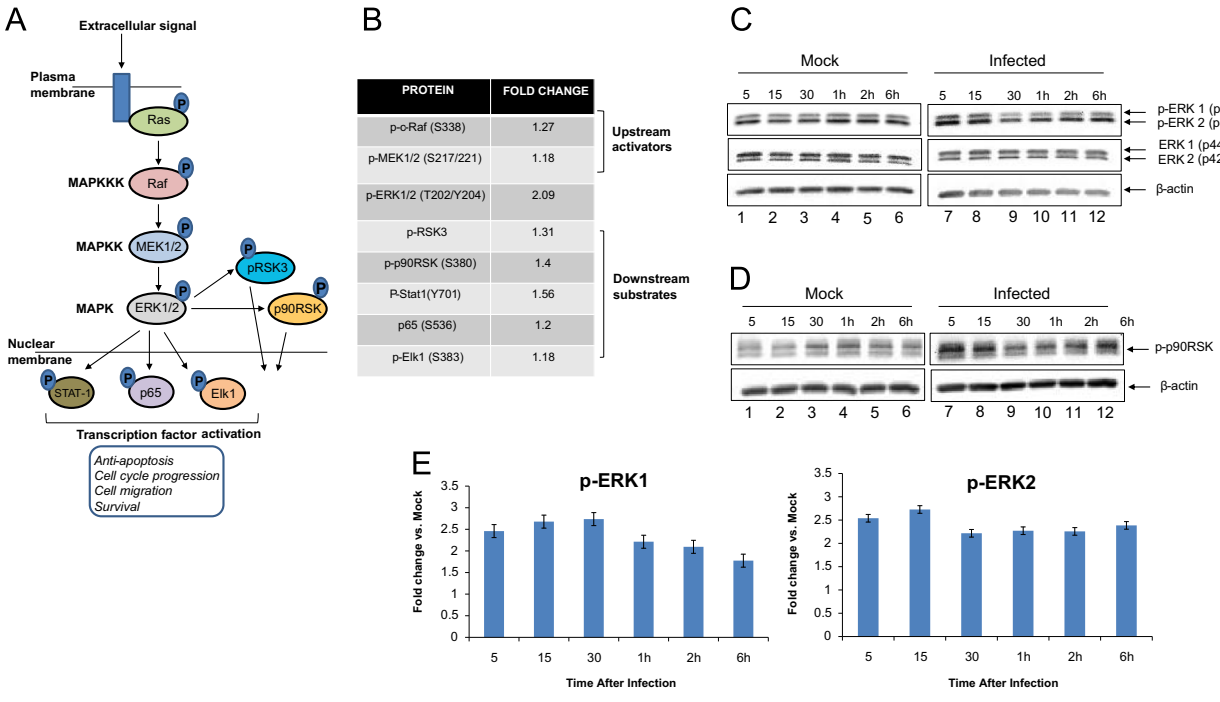

Fig. 1. ERK1/2 signaling is activated in TC-83 infected cells (A) Schematic of ERK signal transduction cascade – upstream activators and downstream targets. (B) Quantification of fold changes in phosphorylation status of target proteins in infected cells over uninfected cells as observed by RPPA. Signal intensities pertaining to phosphorylated forms of proteins were calculated for mock-infected lysates and TC-83 infected lysates following imaging and quantification of the RPPA slides. The values were then used to calculate the fold change in phosphorylation in the infected samples over the mock-infected samples. (C) Western blot validation of ERK phosphorylation in U87MG cells after infection with TC-83. Infected and mock uninfected cell lysates were collected at the time points indicated. β-actin was used as a loading control, and three independent experiments were conducted. (D) Western blot validation of downstream substrate p90RSK phosphorylation. (E) Quantification p-ERK1 and p-ERK2 in infected cells from three independent experiments, normalized to β-actin and then compared to the mock at each time point. Bars indicate the mean and error bars represent standard error.