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Activation of H2AX and ATM in varicella-zoster virus (VZV)-infected cells is associated with expression of specific VZV genes

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ABSTRACT

Mammalian cells activate DNA damage response pathways in response to virus infections. Activation of these pathways can enhance replication of many viruses, including herpesviruses. Activation of cellular ATM results in phosphorylation of H2AX and recruits proteins to sites of DNA damage. We found that varicella-zoster (VZV) infected cells had elevated levels of phosphorylated H2AX and phosphorylated ATM and that these levels increased in cells infected with VZV deleted for ORF61 or ORF63, but not deleted for ORF67. Expression of VZV ORF61, ORF62, or ORF63 alone did not result in phosphorylation of H2AX. While BGLF4, the Epstein-Barr virus homolog of VZV ORF47 protein kinase, phosphorylates H2AX and ATM, neither VZV ORF47 nor ORF66 protein kinase phosphorylated H2AX or ATM. Cells lacking ATM had no reduction in VZV replication. Thus, VZV induces phosphorylation of H2AX and ATM and this effect is associated with the presence of specific VZV genes in virus-infected cells.

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Introduction

DNA damage is induced by numerous stresses to the cell, including ionizing radiation, ultraviolet (UV) radiation, cytotoxic drugs, telomere shortening, programmed breaks (e.g. V(D)J recombination), class switch recombination, and replication stress associated with virus infections (Bonner et al., 2008; Burhans and Weinberger, 2007). Cells respond to DNA damage by activating checkpoint pathways that delay the progression through the cell cycle, promote DNA repair, or induce cell repair (Garner and Costanzo, 2009). Double-stranded DNA breaks are sensed by the MRN complex which results in recruitment and phosphorylation of the tranducers of the DNA damage response, the PI3-kinase-like kinases: ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3-related (ATR), and DNA-dependent protein kinase (DNA-PK) (reviewed in Weitzman et al., 2010). ATM is phosphorylated at serine 1981 (Bakkenist and Kastan, 2003; Lee and Paull, 2005; Carson et al., 2003; Uziel et al., 2003) which in turn phosphorylates many downstream proteins involved in coordinating cell cycle arrest, DNA repair and apoptosis such as H2AX, Mdc1, Nbs1, Chk2, 53BP1, and p53 (Bartek and Lukas, 2003).

H2AX is a member of the histone family which interacts with eukaryotic DNA and helps to regulate transcription. H2AX is phosphorylated on serine 139 to form γ-H2AX at sites of double-stranded DNA breaks, where proteins involved in DNA repair accumulate (Rogakou et al., 1999; Bonner et al., 2008). Phosphorylation of H2AX also occurs with other stimuli including single-stranded DNA breaks induced by ultraviolet radiation and with the normal progression through the cell cycle (Ichijima et al., 2005).

Virus replication presents the host cells with foreign DNA, including free DNA ends and unusual DNA structures. Thus, virus replication induces DNA damage responses which can result in apoptosis as a host defense mechanism against infection (Chaurushiya and Weitzman, 2009). Viruses have evolved a variety of mechanisms to counteract DNA damage responses to enhance replication and persistent infection. Many DNA viruses initially activate the DNA damage response pathway and then subsequently inhibit it (reviewed in Chaurushiya and Weitzman, 2009; Turnell and Grand, 2012; Weitzman et al., 2010). Adenovirus proteins E1B and E4 inhibit the MRN complex (Stracker et al., 2002), while herpes simplex virus (HSV) UL12 associates with the MRN complex and likely facilitates its activity (Balasubramanian et al., 2010). Kaposi sarcoma associated herpesvirus (KSHV) vIRF1 (Shin et al., 2006) and Epstein-Barr virus (EBV) LMP1 (Ma et al., 2011) inhibit ATM, while HSV ICP0 (Li et al., 2008) and cytomegalovirus IE1 (Castillo et al., 2005) activate ATM. EBV BGLF4 (Li et al., 2011) and KSHV v-cyclin (Koopal et al., 2007) activate H2AX.

The human alphaherpesvirus consist of HSV-1, HSV-2, and varicella-zoster virus (VZV). These viruses cause lytic infections

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in epithelial cells and latent infections in neurons of sensory ganglia. HSV and VZV have similar mechanisms of replication in the cell and the seven proteins required for origin-dependent replication of HSV DNA (Challberg, 1986) have orthologs in VZV. HSV and VZV infection of cells brings linear viral DNA with free ends into the cell that resemble double-stranded DNA breaks, as well as nicked viral DNA which has single-stranded DNA breaks. Thus, HSV and VZV infection would be expected to result in activation of the DNA damage response. HSV infection of cells results in replication compartments which contain both viral proteins (ICP8, viral DNA polymerase [Bush et al., 1991; Quinlan et al., 1984]) as well as host cell proteins important for the DNA damage response (Nbs1, Mre11, Rad50, and phosphorylated ATM [Gregory and Bachenheimer, 2008; Lilley et al., 2005; Shirata et al., 2005]). γ-H2AX accumulates in areas surrounding HSV replication complexes (Wilkinson and Weller, 2006). HSV infection results in phosphorylation of other proteins involving the DNA damage response including Nbs1, Chk2, 53BP1, and p53 (Gregory and Bachenheimer, 2008; Lilley et al., 2005; Shirata et al., 2005). While many studies have evaluated the interaction of HSV with DNA damage responses, none have evaluated VZV. Therefore, we studied the effects on VZV infection on activation of cellular proteins involved in the DNA damage response.

Results

Infection with VZV induces phosphorylation of H2AX in melanoma cells and fibroblasts, but not in U2OS cells

HSV-2 has previously reported to induce phosphorylation of H2AX in Vero cells (Wilkinson and Weller, 2006). Therefore we analyzed the effect of VZV infection on H2AX. We found that VZV, like HSV-2, induced phosphorylation of H2AX in melanoma cells (Fig. 1A) or human diploid fibroblasts (Fig. 1B); however, the level of total H2AX was unchanged in virus-infected cells. Phosphorylation of H2AX was about 2-fold higher in melanoma cells and 5-fold higher in human diploid fibroblasts infected with cell-associated VZV ROka than in mock-infected cells. UV-irradiated cells (Fig. 1, lane 1) served as a positive control for phosphorylation of H2AX. A similar increase in phosphorylation of H2AX (4-fold) was observed with parental (wild-type) VZV Oka in human diploid fibroblasts compared with mock-infected cells (data not shown). HSV-2 infection also induced phosphorylation of H2AX in melanoma cells and human diploid fibroblasts.

High levels of phosphorylated H2AX are induced in melanoma cells and fibroblasts after infection with VZV deleted for ORF61 or ORF63, but not deleted for ORF67

Prior studies showed that immediate-early genes of HSV are important in initiating the DNA damage response in HSV-infected cells (Chenet-Monte et al., 1986; Johnson et al., 1992; Peat and Stanley, 1986). Therefore we tested two VZV mutants with deletions, one with a deletion in an immediate-early gene (VZV ORF63) and one with a deletion in a gene homologous to the HSV ICP0 immediate-early gene (VZV ORF61). γ-H2AX levels were 1.6-fold higher in melanoma cells infected with ROka61D (deleted for VZV ORF61) compared with ROka, and 1.7-fold higher in melanoma cells infected ROka63D (deleted for VZV ORF63) than ROka (Fig. 1A). Cells infected with rescued viruses (ROka61DR and ROka63DR) had levels of γ-H2AX closer to ROka. Similar increases in γ-H2AX were also observed in fibroblasts infected with ROka61D and ROka63D (Fig. 1B) and in 293T cells (data not shown).

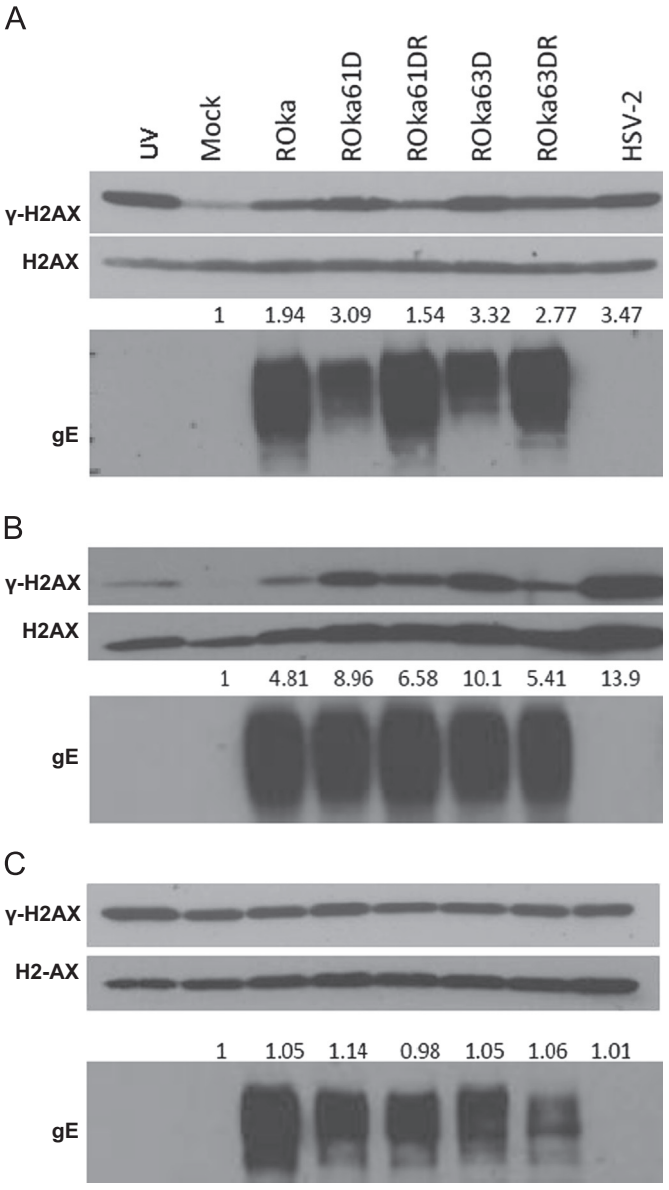

Fig. 1. Immunoblot of γ-H2AX and H2AX expression in melanoma (MeWo) cells (A), human diploid fibroblasts (MRC-5 cells) (B) and U2OS osteosarcoma cells (C) infected with wild-type VZV, VZV mutants, and HSV-2. MeWo, MRC-5 and U2OS cells were infected with 1×10^6 PFU of cell-associated VZV (ROka, ROka61D, ROka61DR, ROka63D, or ROka63DR) or with HSV-2 at an MOI of 0.5. At 48 h after infection with VZV (or 24 h after with HSV-2), the cells were lysed and equivalent amounts of cell lysates were immunoblotted with anti-γ-H2AX, anti-H2AX, and anti-VZV gE antibody. The numbers below the H2AX panels indicate the ratio of intensity of the γ-H2AX and H2AX bands in infected cell lysates divided by the ratio of the intensity of the γ-H2AX and H2AX bands in mock-infected cells. Densitometry was performed using Image J software. UV indicates cells that were treated with 50 mJ/cm^2 of UV irradiation and 14 h later lysates were prepared. The experiments were repeated and similar results were obtained.

U2OS osteosarcoma cells complement the growth of VZV deleted for ORF61 (ROka61D [Cohen and Nguyen, 1998]) and ORF63 (ROka63D [Ambagala and Cohen, 2007]). Therefore, we determined whether phosphorylation of H2AX might be different in ROka63D or ROka61D-infected U2OS cells than in melanoma cells. Levels of γ-H2AX in U2OS were constitutively elevated in the presence or absence of virus infection and at levels similar to the UV irradiated cells (Fig. 1C); therefore, we could not assess the effect of deletion of ORF61 or ORF63 on γ-H2AX in U2OS cells.

Since deletion VZV ORF61 or ORF63 from VZV increased levels of γ-H2AX; we determined whether ORF61 and ORF63 proteins