IRGs, as well as nitric oxide, in the overall mechanism of parasite killing in IFN-γ-activated macrophages? Why is the defect in parasite killing resulting from the silencing of Irgb6 not compensated for by IFN-γ-induced Irgm1 or Irgm3? In addition, because Irgms and other IRGs share the conserved region of Irgb6 targeted by ROP18, why are these GTPases not inactivated by ROP18 by the same mechanism? In this regard, the current and a recent study have reported that ROP18 is indeed also able to phosphorylate Irga6 and Irgb10 (Steinfeldt et al., 2010). A further question concerns whether RPO18 is sufficient and acts as the sole determinant of virulence among the diverse range of *T. gondii* strains and whether other strain-specific kinases expressed in non-type I lineages play a related role.

Interestingly, the remarkable difference in the virulence among the three major types of *Toxoplasma* in mice does not appear to be recapitulated in humans. Moreover, the host targets of ROP18 in mice, Irga6, Irgb6, and Irgb10, are absent in human cells (Hunn et al., 2010), suggesting that this parasite virulence factor may have coevolved with the IRG family. Thus, it appears that ROP18 is utilized by *Toxoplasma* to specifically counteract the effector functions of the IRG family, one of the most powerful host mechanisms for defense against intracellular pathogens documented in nonprimate mammalian species.

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# Keeping Viruses in Chk: DNA Damage Signaling Puts the Brakes on Transformation

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**Oncogenic viruses infect many cells but rarely lead to tumorigenesis. In this issue of *Cell Host & Microbe*, Nikitin et al. describe how a protective DNA damage response acts to suppress transformation in the majority of cells latently infected with Epstein-Barr virus (EBV).**

Although genomic instability is a hallmark of almost all human cancers, in most cases it is still unclear how it arises and contributes to tumorigenesis. In hereditary cancers, germline mutations provide an explanation for the initiation of genome instability, but in sporadic cancers, the molecular basis for the source of instability is often unknown (Negrini et al., 2010). Recent studies in cancer biology have proposed that activation of the DNA damage response (DDR) in early precancerous lesions presents a barrier to tumor progression (Halazonetis et al., 2008). Activated oncogenes induce hyperproliferation and replication stress, resulting in DNA double-strand breaks (DSBs) that induce the DDR (Figure 1). This response activates the p53 tumor suppressor and triggers cell-cycle arrest, senescence, or apoptosis. The DDR in precancerous lesions thereby acts as an inducible barrier against genomic instability, restricting tumorigenesis (Bartkova et al., 2005; Gorgoulis et al., 2005). This imposes a selective pressure for acquisition of mutations that compromise the checkpoint, and during cancer progression these defects result in suppression of signaling, facilitating escape from apoptosis and senescence (Halazonetis et al., 2008).

The DDR represents a cellular surveillance network of signaling pathways that

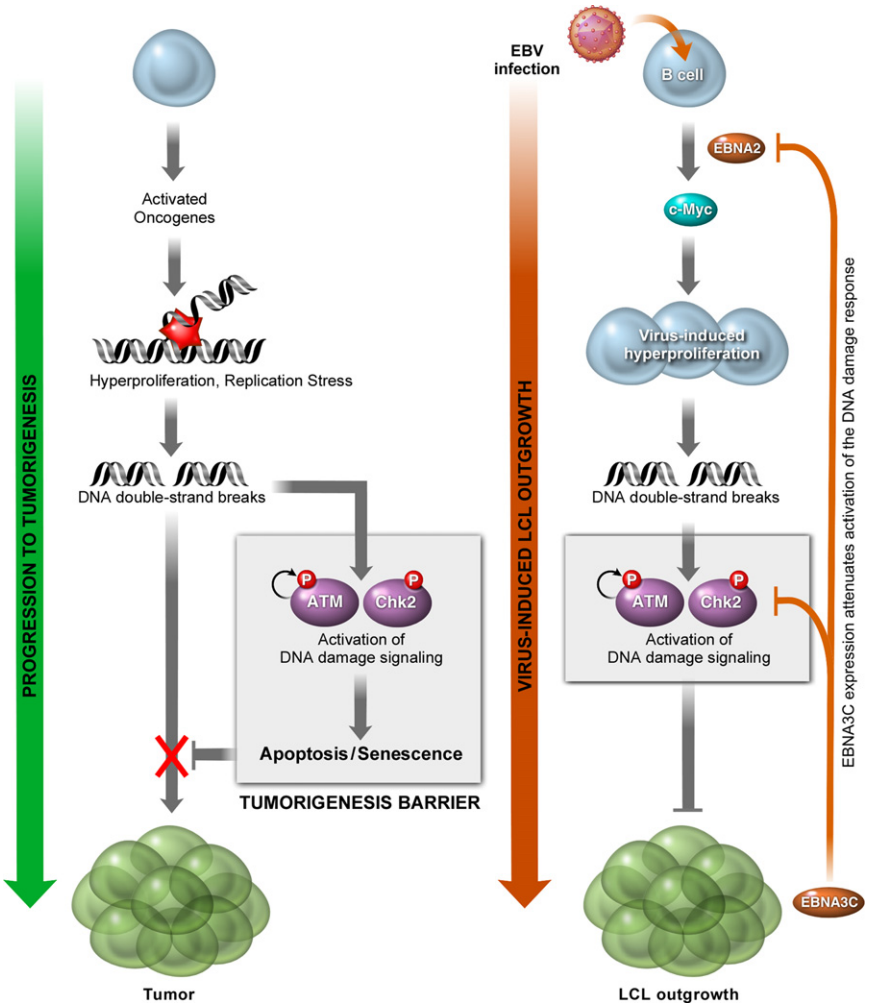


**Figure 1. The DNA Damage Response Is an Anticancer Barrier that Restricts Transformation in EBV-Infected Cells**

In the oncogene-induced DNA damage model of cancer development, activated oncogenes induce stalling and collapse of DNA replication forks. The resulting DSBs generate a cellular DNA damage response (DDR), characterized by kinase phosphorylation and checkpoint activation. This response leads to cell-cycle arrest, senescence, and apoptosis, preventing cells from progressing to tumors (left side). In this way, the DDR in precancerous lesions acts as an inducible barrier (boxed), restricting tumorigenesis. In the majority of B cells latently infected with EBV, EBNA2 and latency III gene expression induces activation of downstream oncogenes and an early period of hyperproliferation. The resulting DDR restricts long-term outgrowth in the majority of cells. During LCL outgrowth, this DDR is attenuated by EBNA3C, acting either directly on the DNA damage machinery or indirectly, by downregulating the activity of EBNA2.

sense the damage and sound alarms to direct decisions between repair and apoptosis (Jackson and Bartek, 2009). When cells experience replication stress or encounter DNA damage lesions, cell-cycle checkpoints are activated to prevent replication and segregation of damaged genomes. The DDR kinase ATM phosphorylates a large number of protein substrates, including p53, to activate checkpoints and coordinate apoptosis. ATM also directly phosphorylates the checkpoint kinase Chk2 and mediates tumor suppression in response to DNA damage. Activation of these DDR markers is observed in precancerous lesions, and continuous production of DSBs can be detected prior to acquisition of p53 mutations (Bartkova et al., 2005; Gorgoulis et al., 2005). Therefore, these kinases and their substrates serve as the gatekeepers of the tumorigenesis barrier, and cancer develops when the barrier is breached through their inactivation.

Epstein-Barr virus (EBV) is linked to a number of human malignancies. Although multiple viral proteins have been implicated in promoting genomic instability (Gruhne et al., 2009), little is known about the cellular factors that govern the outcome of infection. In this issue of *Cell Host & Microbe*, a paper by Nikitin and coworkers demonstrates that latent EBV infection induces a cellular DDR that acts to limit viral-mediated transformation (Nikitin et al., 2010). In culture, EBV infection drives primary human B cells into indefinitely proliferating lymphoblastoid cell lines (LCLs). An initial wave of expression from viral and cellular genes leads to proliferation of infected cells, but only a small percentage of cells progress to become LCLs. In these cells, EBV establishes a latent infection, characterized by expression of a limited subset of viral genes. These include Epstein-Barr nuclear antigens (EBNAs) 1, 2, 3A, 3B, 3C, leader protein (EBNA-LP) and latent membrane proteins (LMPs) 1, 2A, and 2B. Only EBNAs 1, 2, and 3C and LMP1 are essential for transformation of B cells. Different stages of EBV latency have been defined, and expression of EBNAs 2, 3, 4, 5, and 6 characterizes the most advanced stage, termed latency III. Nikitin et al. examined latent infections of primary B cells by a transforming strain of EBV and observed activation of checkpoint markers (Figure 1). Induction of the EBV-induced DDR was not due to lytic gene expression or latent EBV DNA but required EBNA2 and latency III gene expression. Signaling correlated with a virally induced state of cellular hyperproliferation at early times postinfection, reminiscent of the DDR activated by S phase-promoting cellular oncogenes (Bartkova et al., 2005).

If the DDR does indeed act as an anticancer barrier active in early tumorigenesis, oncogenic viruses that induce transformation must be able overcome this barrier. In the present study, Nikitin et al. suggest that the EBV-induced DDR is attenuated during outgrowth of the LCLs. Microarray studies confirmed that an ATM-dependent p53 target signature was induced in EBV-infected cells, but that activated genes were subsequently repressed in the transition from early proliferating cells to established LCLs. Analyzing viral gene expression at various times postinfection, Nikitin et al. detected a shift in the relative ratio of viral proteins as LCL outgrowth began. The switch

in equilibrium of EBNA gene expression would be expected to induce changes in expression of host target genes. This was seen with EBNA2 targets, which were highly induced in the early cell divisions after infection but were subsequently attenuated through LCL outgrowth. Multiple EBV proteins have been suggested to affect cellular DNA damage pathways (Gruhne et al., 2009). In the current study, infections with virus lacking EBNA3C demonstrated that this viral protein was required for attenuation of the host DDR (Nikitin et al., 2010), in keeping with its elevated expression during LCL outgrowth. EBNA3C displays a plethora of diverse functions that could contribute to the diminution of the DDR during LCL outgrowth, including inhibition of EBNA2-activated transcription, downregulation of Chk2, and repression of p53 activity. Therefore, as EBV attenuates the genotoxic and growth-suppressive signaling pathway it has induced, the outgrowth of genetically stable LCLs is enabled. This model highlights how dysregulation of host or EBV latent gene expression could lead to tumorigenesis. A critical balance between latent oncoprotein-driven hyperproliferation, host gene expression, and stable proliferative signals in LCLs is required to maintain the activated, immortalized state.

Since ATM and Chk2 are known to be important regulators of the DNA damage antitumor response, Nikitin et al. explored whether inhibiting these kinases allowed the virus to bypass the protective barrier. Strikingly, when PBMCs were simultaneously infected with EBV and treated with small-molecule inhibitors of ATM or Chk2, transformation efficiency increased. EBV-infected cells were most sensitive to the inhibitors during the hyperproliferative period, indicating that an early period of growth suppression mediated by the DDR restricts transformation.

The paper by Nikitin et al. raises a number of questions. Among these are the mechanism by which EBNA2 activates the DDR and whether it requires additional viral proteins. A hint comes from the observation that S phase-promoting oncoproteins are among the targets of EBNA2. For example, c-Myc and the genes it activates were highly induced in early cell divisions and then attenuated through LCL outgrowth. Since ATM is known to be involved in suppressing c-Myc oncogenesis, these data support the hypothesis that it is acute oncogenic stress early after EBV infection that leads to hyperproliferation and DNA damage signaling. The mechanism and rationale for EBNA3C attenuating DNA damage signaling as LCLs emerge also remains to be determined. It is possible that EBNA3C regulates EBNA2 induction of the DDR to prevent viral clearance or apoptosis, and any effects on the efficiency of transformation are merely a secondary outcome. Furthermore, even with ATM and Chk2 inhibitors, the transformation efficiency of EBV-infected PBMCs remains low, suggesting that additional mechanisms exist to restrict viral-mediated transformation.

It was previously known that lytic EBV infection induces ATM activation and phosphorylation of downstream substrates. Cellular DNA damage proteins accumulate in viral replication compartments, but preventing their activation does not overtly affect viral replication (Kudoh et al., 2005). The current study focuses on latent EBV, adding an additional layer of complexity to the interaction of this virus with the cellular DNA damage machinery. Like EBV, many viruses have evolved complex and multifaceted relationships with the cellular DNA damage proteins (Lilley et al., 2007), highlighting the fundamental nature of this interface. The related oncogenic herpesvirus, Kaposi's sarcoma herpesvirus (KSHV), has a strikingly similar interaction with the DDR pathway (Koopal et al., 2007). Like EBV, oncogenic stress caused by a KSHV latent viral protein induces DNA damage signaling and antiproliferative checkpoints in an early population of hyperproliferating cells. Other transforming viruses such as the human T-lymphotropic virus (HTLV-1) and adenovirus also encode proteins that promote hyperproliferation and activate DNA damage markers (Lilley et al., 2007). It will be interesting to see if the innate tumor suppressor function proposed for the DDR in the current study is a paradigm for regulation of transformation by other oncogenic viruses and whether these observations in cell culture are relevant to viral-induced tumorigenesis in vivo.

In conclusion, the study by Nikitin et al. demonstrates that the oncogene-induced DNA damage model for cancer development is not restricted to controlling aberrant cellular tumorigenic events. It raises the possibility that this anticancer pathway may have evolved, at least in part, as a protective mechanism against viruses. This study presents another example of the intricate balance achieved by viruses in their interactions with protective host pathways and also highlights the importance of controlled temporal feedback to regulate viral functions as infection progresses.

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