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Take your PIKK: tumour viruses and DNA damage response pathways

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Viruses regulate cellular processes to facilitate viral replication. Manipulation of nuclear proteins and pathways by nuclear replicating viruses often causes cellular genome instability that contributes to transformation. The cellular DNA damage response (DDR) safeguards the host to maintain genome integrity, but DNA tumour viruses can manipulate the DDR to promote viral propagation. In this review, we describe the interactions of DNA tumour viruses with the phosphatidylinositol 3-kinase-like protein kinase (PIKK) pathways, which are central regulatory arms of the DDR. We review how signalling through the ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3 related (ATR), and DNA-dependent protein kinases (DNA-PK) influences viral life cycles, and how their manipulation by viral proteins may contribute to tumour formation.

This article is part of the themed issue 'Human oncogenic viruses'.

1. Introduction

Viruses are obligate, intracellular pathogens that manipulate host cells to establish environments conducive to viral replication. Interference with nuclear processes by viruses often results in unchecked cellular division, which contributes to cellular transformation and cancer. Current estimates indicate that at least 10% of all human cancers worldwide are caused by viruses [1]. Viruses associated with tumour formation—'tumour viruses'—include human papillomavirus, Merkel cell polyomavirus, Kaposi's sarcoma-associated herpesvirus, Epstein–Barr virus, human T-lymphotropic virus 1, and hepatitis B and C viruses. Additionally, adenovirus and the polyomavirus simian virus 40 (SV40) can promote tumour formation in rodents [2–5] and transform human cells in culture [6,7], although they are not associated with human cancers. Studying the manipulation of nuclear processes by these tumour viruses has revealed fundamental principles of cellular transformation [6–10].

Maintenance of cellular genome integrity is paramount to preventing cellular transformation. Thus, cells have a plethora of mechanisms in place to preserve genome integrity. The pathways activated by DNA damage to protect genome integrity are collectively called the DNA damage response (DDR), and they function to sense and repair damage in cellular DNA (reviewed in [11–14]). The DDR also responds to viruses, which trigger DDR activation through several means [15]. For example, viral genomes and replication intermediates may activate the DDR due to their resemblance to damaged DNA structures. In addition, rapid viral DNA replication may cause replication stress or errors that trigger the DDR. Viral inactivation of cell-cycle checkpoints may also allow mutations to accumulate in cellular DNA. Activation of the DDR during infection can have a myriad of consequences for virus replication, and viruses therefore manipulate the DDR to promote infection. Other excellent reviews have described in detail the ways in which DNA and RNA viruses interact with various components of the DDR [15–19]. In this review, we focus primarily on the mechanisms by which DNA tumour viruses manipulate DDR signalling through the phosphatidylinositol 3-kinase-like protein kinase (PIKK) family. Kinases from

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this family are central regulators of the DDR, and viral manipulation of their signalling pathways can have profound effects on cellular genome integrity. We highlight the resulting consequences of viral manipulation for both viral replication and host genome integrity.

2. DNA damage response

Cellular genomes are damaged on average 100 000 times per day [11]. Sources of damage include exogenous assaults such as radiation, and endogenous events such as replication fork collapse and DNA replication errors. DNA damage occurs in multiple forms, including mismatched base pairs, pyrimidine dimers, replication stress, and single-strand or double-strand DNA breaks [11]. Unchecked DNA damage has dramatic effects on cells since the accumulation of mutations and DNA breaks can lead to cell death, chromosomal translocations and oncogenesis.

The DDR is a network of signal transduction pathways that respond to DNA damage. Signalling is mediated by serine/threonine kinases within the PIKK family and the downstream proteins that are activated [11–14]. DDR signalling leads to arrest of the cell cycle to allow recruitment of proteins to repair the damaged DNA [11–14]. Alternatively, signalling can induce apoptosis to eradicate the damaged cell. The DDR is activated by recognition of DNA damage via proteins called 'sensors'. Sensors bind DNA at the site of damage and recruit PIKK 'transducers'. Transducers in turn activate multiple downstream 'effectors' to amplify signalling that mediates DNA repair and cell-cycle arrest at the G1/S, intra-S and G2/M checkpoints [11–14]. Effectors include tumour suppressors, which halt cell division by activating cell-cycle checkpoints or apoptosis. Loss or inhibition of tumour suppressors can lead to unregulated cellular proliferation and transformation. Viruses regulate the DDR through manipulation of proteins at all three stages of the DDR.

The primary transducers of the DDR are ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3 related (ATR), and DNA-dependent protein kinase (DNA-PK). ATM, ATR and DNA-PK are all members of the PIKK family and have similar domain structures, including kinase and protein-binding domains [20,21]. The specific PIKK activated depends on the type of DNA damage encountered. ATM and DNA-PK respond to double-strand DNA breaks (DSBs), while ATR responds to replication stress and single-stranded DNA (ssDNA) [20,21]. The specific proteins activated in each pathway are illustrated in figure 1. Briefly, the MRE11-RAD50-NBS1 complex (MRN) senses DSBs and promotes activation of ATM [22,23]. ATM is activated by autophosphorylation and through interactions with TIP60 [24,25], and activated ATM then phosphorylates downstream effectors to amplify signalling. Effectors include histone H2A variant H2AX (γH2AX when phosphorylated), NBS1, BRCA1, CHK2 and p53 [26–31]. BRCA1 and RAD51 are required for repair of DSBs by homologous recombination during S-phase, and CHK2 and p53 activate the G1/S, intra-S and G2/M checkpoints [30–33]. Another repair pathway for DSBs is non-homologous end joining (NHEJ), which requires DNA-PK activity. The Ku complex senses DSBs and recruits the catalytic subunit of DNA-PK (DNA-PKcs) [11]. The Ku-DNA-PKcs complex recruits XRCC4 and DNA ligase IV to join broken ends [34]. Accumulation of ssDNA at resected DSBs and replication forks promotes activation of the ATR pathway. Exposed ssDNA is coated and protected by RPA, which recruits ATR through the ATR binding partner ATRIP [35]. The ATR activator TOPBP1 is recruited by interacting with the 9-1-1 complex (RAD9, RAD1, HUS1) [36]. ATR activation signals through downstream effectors CHK1 and p53 to cause cell-cycle arrest at the G2/M and intra-S checkpoints or apoptosis [32]. Since cell-cycle arrest or cell death could limit viral replication, all of the tumour viruses discussed here employ multiple strategies to misregulate the cell cycle, most notably through inactivation of tumour suppressors p53 and RB [6–10].

Misregulation of the cell cycle via disruption of tumour suppressors is a significant contributor to transformation by tumour viral oncoproteins and has been described in other reviews [6–10]. Here, we instead summarize viral regulation of upstream PIKK signalling through ATM, ATR and DNA-PK. We describe DNA tumour viruses from several viral families to highlight the diverse interactions that viruses can have with the PIKK pathways.

3. Adenovirus

The intricate relationship between viruses and the DDR has been extensively demonstrated with adenovirus serotype 5 (Ad5). All three of the PIKKs are targeted by adenoviral proteins, and these interactions revealed principles that have since been extended to other viruses. Adenovirus has a linear, double-stranded DNA genome, and one of the first indications that the DDR responded to adenovirus was the observation that infection with genetic mutants resulted in fusion of viral genomes into concatemers [37]. This observation led to the hypothesis that the blunt, double-stranded DNA ends of the Ad5 viral genome are recognized as DNA breaks. Several DDR proteins are necessary for concatemer formation, supporting a role for the DNA repair machinery [38,39]. This was the first demonstration that the cellular DDR recognizes and acts on viral DNA. While the DDR responds to mutant adenovirus infection, wild-type Ad5 infection does not produce concatemers [23,37,38], indicating that Ad5 evades the DDR. Inactivation of DDR components is critical for efficient Ad5 replication [39–43], suggesting a role for the DDR in restricting adenoviral replication.

(a) The MRE11-RAD50-NBS1 complex

The cellular MRE11, RAD50 and NBS1 proteins comprise the MRN complex (MRN), which can act as a sensor of double-strand DNA breaks (figure 1). Ad5 regulates MRN localization and protein levels to minimize the impacts of host detection of viral DNA. During Ad5 infection, early viral proteins both degrade MRN and mislocalize MRN into nuclear tracks and perinuclear aggresomes [38,41,42,44–49]. The MRN proteins become immobilized, preventing localization to Ad5 replication centres [38,50]. Mislocalization is also necessary for SUMOylation of MRE11 and NBS1 by an early viral protein, although the consequences of SUMOylation are unclear [51].

Evading MRN appears to be important for the Ad5 life cycle. In the absence of MRN mislocalization or degradation, MRN is present at viral replication centres where it associates with viral DNA in an NBS1-dependent manner [38,52,53]. Ad5 mutants unable to target MRN are severely impaired in viral DNA replication, late protein expression and virion production [40,41,53]. Although MRN is required for

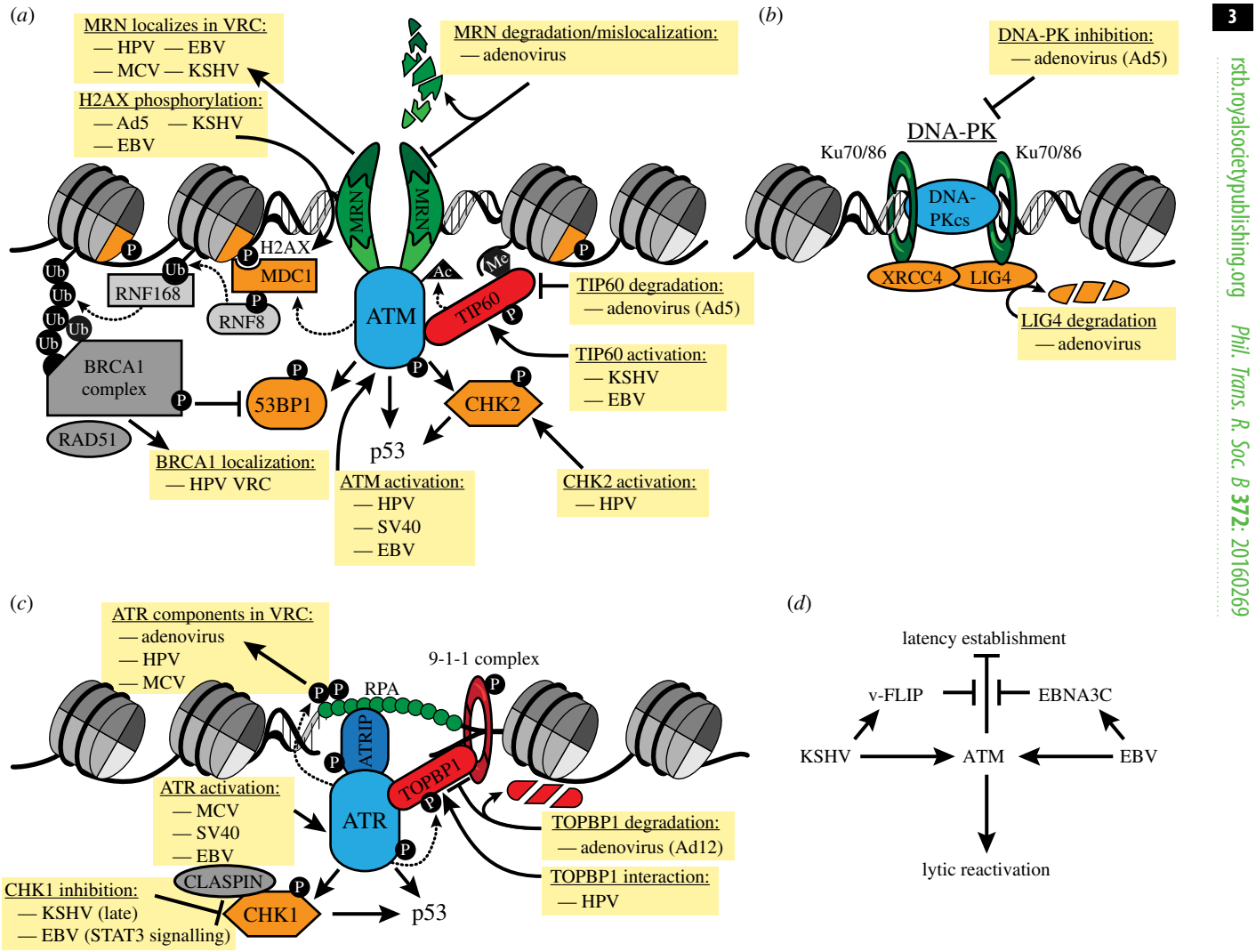

Figure 1. Interactions between DNA tumour viruses and the DNA damage response. DNA tumour viruses manipulate or harness PIKK signalling for viral benefit. (*a*) ATM signalling is activated in response to double-strand DNA breaks (DSBs). The MRN sensor responds to DSBs and activates ATM phosphorylation of downstream substrates. MRN can be targeted by some viruses (e.g. adenovirus), while other viruses recruit the complex to viral replication centres (VRC). Some viruses activate and harness ATM signalling for efficient infection. (*b*) Signalling through DNA-PK is activated by recognition of DSBs by the Ku70/Ku80 complex and results in DNA repair by non-homologous end joining. Adenovirus suppresses the DNA-PK pathway in multiple ways. (*c*) The ATR pathway responds to prolonged exposed single-stranded DNA. Adenovirus, Kaposi's sarcoma-associated herpesvirus (KSHV) and Epstein-Barr virus (EBV) inhibit components of the ATR pathway, but ATR proteins associate with replication centres of several viruses. (*d*) The γ-herpesviruses KSHV and EBV activate the ATM pathway for lytic reactivation, but inhibit ATM to establish latency. Examples of viral proteins known to target these pathways are indicated.

concatemers [38], MRN can also impair viral replication independently of concatemer formation [40,44,53]. Loss of MRN rescues replication of Ad5 mutants that neither mislocalize nor degrade MRN [40,53]. Ad5 mutants that target MRN by only one of these mechanisms are not impaired for viral replication, demonstrating that each mechanism is sufficient to evade MRN [40].

There are several potential models for MRN restriction of adenovirus replication. One model is that MRE11 removes the viral terminal protein (TP) from the 5′ ends of the adenovirus genome through its nuclease activity. TP provides the 3′ hydroxyl group to initiate DNA replication and may protect viral DNA from digestion, so its removal would have a profound effect on adenovirus replication. This model is supported by the loss of DNA sequences at concatemer junctions and the requirement for MRE11 exonuclease activity for concatemer formation [37,38,48]. Alternatively, recruitment of DDR proteins to viral DNA could physically obstruct the interaction of viral and cellular replication proteins with viral genomes [54]. A third model is that MRN indirectly impairs replication through activation of downstream ATM signalling, which is supported by enhanced viral replication during ATM inhibition [42,43]. While it is clear that MRN is a major obstacle for Ad5 replication, the mechanism by which it restricts replication requires further study.

The interaction of Ad5 with MRN is not representative of all adenovirus serotypes. While Ad5 and closely related serotypes target MRN through both degradation and mislocalization, other serotypes target MRN through only one mechanism, and some do not target MRN through either mechanism [46,47,55,56]. It remains to be determined how these differences influence viral replication and whether MRN inactivation can occur by alternative mechanisms.

(b) Ataxia telangiectasia mutated kinase

Observations from Ad5 were the first to demonstrate that MRN promotes ATM activation in response to viruses and cellular double-strand breaks in mammalian cells [23]. Since MRN is the sensor that activates ATM signalling, it would

be expected that MRN targeting by Ad5 abrogates ATM activation. Multiple groups have observed that degradation of MRN by wild-type Ad5 can prevent activation of ATM or downstream substrates at viral replication centres [23,42,43]. Ad5 also employs means to prevent ATM activation before viral proteins are expressed. Protein VII, a viral core protein bound to incoming viral DNA, is negatively correlated with phosphorylated ATM on mutant Ad5 genomes early during infection [54]. This suggests a role for protein VII in preventing DDR recognition of incoming viral genomes. Another mechanism by which Ad5 may regulate ATM activation is through degradation of the ATM activator TIP60 [57]. Together, these studies demonstrate multiple ways that Ad5 infection can affect ATM activation and signalling.

While there is consensus that ATM is not activated during early infection or at wild-type Ad5 replication centres, some findings demonstrate pan-nuclear distribution of activated ATM late in infection [42], suggesting MRN-independent ATM activation during virus infection. This is consistent with the reported phosphorylation of the ATM substrate KAP1 and replication-dependent widespread γH2AX during wild-type Ad5 infection [42,46,58]. KAP1 phosphorylation is also seen during infection with other Ad serotypes [46].

The effect of ATM activation on Ad5 infection may vary between cell types and stages of the viral life cycle. When viral replication was measured by quantitative PCR in ATM hypomorphic fibroblasts, ATM loss did not enhance replication of an Ad5 mutant unable to target MRN [40]. However, when viral replication was measured by dot blot hybridization in transformed cell lines, increased viral DNA from the mutant Ad5 was observed when ATM was inhibited or depleted [43]. In primary lung epithelial cells, ATM has distinct effects on Ad5 at different stages of replication [42]. An Ad5 mutant incapable of targeting MRN was impaired by ATM activation at replication centres early during infection in small airway epithelial cells [42]. In these cells, wild-type Ad5 avoided ATM activation at replication centres by targeting MRN and progressed to late infection when diffuse ATM activation occurred. Inhibition of ATM kinase activity during wild-type Ad5 infection does not affect replication in transformed or primary cells [42,43]. Together, these findings suggest that ATM does not impair wild-type Ad5 but may inhibit replication of specific Ad5 mutants in various cellular settings.

(c) Ataxia telangiectasia and Rad3 related kinase

ATR signalling is also abrogated during adenovirus infection. While ATR is generally associated with prolonged exposure of ssDNA due to replication stress, double-strand breaks can induce ssDNA exposure and subsequent ATR activation due to MRE11-mediated resection at broken ends [59]. Ad5 mutants that do not target MRN induce robust activation of ATR signalling [23,50], which could occur due to replication intermediates or resection at genome ends. ATR activation is prevented during infection with wild-type Ad5 due to MRN degradation and mislocalization [23,46,50]. ATR and several downstream proteins are found at viral replication centres [50,60] but ATR does not appear to affect Ad5 replication [40,42,43]. Adenovirus serotype 12 (Ad12) inhibits ATR through degradation of the ATR regulator TOPBP1 [61]. Interestingly, Ad12 does not mislocalize MRN and therefore does not inhibit ATR through this mechanism [55]. It appears that Ad12 and Ad5 employ distinct mechanisms to inhibit ATR, while some other adenovirus serotypes induce robust ATR signalling [46]. Inactivation of ATR by adenoviruses may simply be a downstream consequence of MRN manipulation, or it may be specifically targeted to promote some undetermined aspect of the life cycle.

(d) DNA-dependent protein kinase

The formation of adenoviral genome concatemers requires DNA-PK and NHEJ proteins to ligate DNA ends, and correlates with decreased late protein expression and DNA packaging [39,62]. Adenovirus proteins overcome these limitations by disabling the DNA-PK pathway. All adenovirus serotypes examined to date degrade DNA ligase IV [46,47,63], and Ad5 early proteins also interact with DNA-PK to inhibit its functions [39].

(e) Summary

Adenovirus has been a powerful model to uncover fundamental principles of virus-host interactions, including interactions with the DDR. Studies with adenovirus were the first to demonstrate that the host DDR responds to viral DNA. In the case of adenovirus, DDR proteins seem to be inhibitory, and adenoviruses thus disable DDR pathways to overcome anti-viral defence and promote viral replication.

An important remaining question is how such extensive dismantling of the host DDR during infection affects cellular genome stability. Adenovirus inactivates cell-cycle checkpoints and all three PIKK pathways, allowing for uncontrolled cellular division without intact DNA repair pathways. This presumably leads to accumulation of genomic instability, yet adenovirus is not associated with any human cancers. It has been suggested that adenovirus may transform cells through a 'hit-and-run' mechanism, where the transforming agent promotes transformation but is not maintained in the cell [64]. However, the most probable barriers to adenovirus-induced oncogenesis in humans are cell death and immune responses that result from productive adenovirus replication. The effect of adenovirus infection on cellular genome stability remains to be determined, but it is clear that the DDR is a major obstacle that must be overcome to establish adenovirus infection.

4. Human papillomavirus

Human papillomaviruses (HPV) are small DNA viruses with circular double-stranded genomes. High-risk HPV types are the etiologic agents of almost all cervical cancers and are associated with anogenital and oropharyngeal cancers. HPV oncogenesis results from expression of viral oncoproteins that inactivate tumour suppressors to induce cellular proliferation [8]. Additionally, viral genomes sometimes integrate into the host genome, causing genome instability and subsequent oncogenesis [8]. The HPV life cycle is linked to the differentiation status of host keratinocytes [65]. HPV infects undifferentiated keratinocytes in the basal layer of the epidermis, where the initial amplification phase of replication produces 20–100 genome copies per cell. Viral episomes are maintained at these levels in dividing, undifferentiated cells during the maintenance phase of the life cycle. During this phase, viral DNA is replicated in synchrony with cellular DNA and distributed to daughter cells through interaction with mitotic chromosomes [66]. As infected cells detach from the basal layer and

differentiate, the virus enters the vegetative replication phase, when late protein expression and virion production occur.

Unlike adenovirus, the circular genome of HPV does not have any exposed DNA ends that could be recognized as DNA breaks. However, multiple events during the HPV life cycle generate substrates that could activate the DDR. Rapid accumulation of viral DNA during the vegetative replication phase of the HPV life cycle causes replication stress that could trigger the ATR pathway. In addition, the transition from maintenance to vegetative replication may be accompanied by rolling circle replication [67], which could expose double-stranded DNA ends. Onion-skin replication from integrated HPV DNA produces replication intermediates that could also trigger DDR activation [68]. Several groups have demonstrated that HPV activates multiple PIKK pathways. While adenovirus suppresses DDR activation, HPV instead benefits from activation by exploiting DDR proteins to promote viral replication at different stages of its life cycle.

(a) Ataxia telangiectasia mutated kinase

Observations from several groups demonstrate that HPV, or expression of HPV proteins, can activate ATM signalling. ATM signalling is activated in cells containing extrachromosomal genomes of high-risk HPV types [69,70], and ATM pathway proteins localize to HPV replication foci [69–72]. Homologous recombination proteins RAD51 and BRCA1 increasingly colocalize with HPV replication foci as viral amplification progresses, suggesting a role for homologous recombination in HPV replication [71]. Expression of viral replication protein E1 is sufficient to activate ATM and CHK2 and promote γH2AX foci [70,72,73]. The viral oncoprotein E7, which regulates the cell cycle, interacts with ATM and is sufficient to activate CHK2 [69]. E7 may also influence ATM phosphorylation through activation of the ATM regulator STAT-5 [74]. The robust activation of ATM signalling in response to HPV genomes or proteins suggests a role for ATM in viral replication.

The consequence of ATM activation for HPV replication depends on the stage of the HPV life cycle. In undifferentiated cells, neither inhibition of ATM nor CHK2 affects episome stability or transient HPV DNA replication [69,72,73,75]. These observations suggest ATM signalling does not affect initial amplification or maintenance. However, ATM depletion reduces episome levels [75], suggesting that ATM may affect DNA replication through other pathways. In differentiated cells, inhibition of ATM and CHK2 reduces the number of HPV replication foci and decreases the level of HPV episomal DNA [69]. Consistent with this finding, depletion of STAT-5 suppresses ATM and CHK2 activation and decreases episome levels in differentiated cells [74]. Together, these observations demonstrate that the ATM-CHK2 pathway promotes HPV replication in differentiated cells.

(b) The MRE11-RAD50-NBS1 complex

MRN also promotes HPV replication in differentiated cells. MRN protein levels increase upon E7 expression, and E7 interacts with NBS1 and RAD50 [76]. MRN colocalizes with HPV DNA, and depletion of NBS1 prevents MRN localization to viral DNA [76]. NBS1 depletion results in decreased episome levels in differentiated cells but has no effect in undifferentiated cells [76]. This implicates MRN in productive HPV replication, consistent with the proposed role for ATM.

(c) Ataxia telangiectasia and Rad3 related kinase

Rapid viral replication during vegetative replication may lead to replication stress that would activate the ATR pathway. Consistent with viral replication stress, proteins within the ATR pathway localize to HPV replication centres [68,71,72]. The amount of RPA32 at replication foci increases with differentiation [71], suggesting a response to increased viral replication. Viral E2 interacts with ATR activator TOPBP1, and disruption of this interaction diminishes episome levels, suggesting a role for TOPBP1 in episome maintenance [77]. Depletion of ATR or inhibition of downstream CHK1 significantly reduces episome stability, implicating the ATR pathway in episome maintenance [75].

(d) Summary

Rather than inactivating the DDR, it seems that HPV evolved to harness the DDR for viral replication. DDR activation normally leads to cell-cycle arrest, but HPV oncoproteins prevent downstream checkpoint activation through disruption of tumour suppressors [8]. This facilitates transformation and may also contribute to the accumulation of mutations and chromosomal aberrations observed in HPV-infected cells. Genome instability is required for HPV-induced malignancy [8]. Given the importance of DDR pathways in vegetative HPV replication, inhibition of the DDR could be used to suppress HPV replication in infected patients during this phase to prevent subsequent integration or transformation.

5. Merkel cell polyomavirus

Merkel cell polyomavirus (MCV) is a small DNA virus with a circular double-stranded DNA genome. Integrated MCV genomes have been implicated in Merkel cell carcinoma (MCC), an aggressive skin cancer [78]. MCV shares a similar genome structure with other polyomaviruses. The viral large tumour antigen (LT) has several roles in MCV replication, including manipulation of the cell cycle and initiation of viral DNA replication [79]. However, in MCC tumours the LT protein expressed from integrated genomes is truncated such that C-terminal domains are absent [80].

(a) Ataxia telangiectasia mutated, and ataxia telangiectasia and Rad3 related kinases

The ATM and ATR pathways are both activated in MCV-infected cells [81]. Several downstream effectors localize to MCV replication foci in response to viral DNA replication, and ATM and ATR kinase activity promote viral replication [82]. Expression of LT is sufficient to activate signalling through ATR but not ATM [82], although the implications of this selective activation are unknown. LT expression also activates p53 transactivation and cell-cycle arrest through its C-terminus in an ATR-dependent manner [82]. This function of LT is contrary to those of LT from other polyomaviruses, which inhibit p53 [7], and suggests that truncation of MCV LT in MCC allows for uncontrolled cellular proliferation. In addition, LT is itself a substrate for phosphorylation by ATM, and this may contribute to the anti-proliferative function of LT [83].

(b) Summary

While research into the interaction of MCV with the DDR has only recently begun, it is apparent that DDR components respond to MCV DNA and promote viral replication. Unlike LT from other polyomaviruses, MCV LT activates cell-cycle arrest. This function of LT is lost in MCC cells, which express a truncated LT. This observation provides insight into the mechanism behind MCV-mediated transformation and could be exploited to target MCC cells.

6. Simian virus 40

Simian virus 40 (SV40) is a polyomavirus with a circular double-stranded DNA genome that naturally infects rhesus macaques. Like MCV and other polyomaviruses, the SV40 genome expresses large T (LT) and small T (sT) antigens to regulate viral replication. While SV40 infection causes tumours in newborn hamsters [4,5], and SV40 LT and sT can transform human cells in culture [7], there is no evidence of SV40-mediated oncogenesis in humans or macaques.

(a) Ataxia telangiectasia mutated, and ataxia telangiectasia and Rad3 related kinases

SV40 infection causes activation of the ATM and ATR pathways. Downstream effectors of these DDR kinases are located at foci in response to LT expression or SV40 infection [84–89]. Activation of the ATM pathway is dependent on LT binding to the spindle checkpoint protein BUB1 [86], and LT itself is phosphorylated by ATM [84]. SV40 activation of ATM results in downstream phosphorylation of p53 [86], but p53 transactivation is in turn inhibited by LT to prevent p53-mediated cell-cycle arrest [7].

Activation of the DDR promotes SV40 replication. Inhibition or depletion of ATM results in decreased viral replication [84,85]. ATM promotes viral replication through the downstream cohesin protein SMC1 and phosphorylation of LT [84,85]. Similarly, ATR inhibition results in decreased viral DNA levels [89]. ATM and ATR inhibition were found to induce accumulation of aberrant structures and stalled replication forks in viral DNA [89]. Inhibition of RAD51 also reduces viral DNA levels, suggesting a role for homologous recombination in SV40 DNA replication [88].

(b) The MRE11-RAD50-NBS1 complex

SV40 targets MRN to enhance viral replication. NBS1 suppresses SV40 DNA replication and prevents inappropriately timed cellular DNA replication. LT interacts with NBS1 to enhance viral replication [90], and this interaction was suggested to cause the endoreduplication and hyperploidy observed in SV40-infected cells [91]. Furthermore, SV40 has been suggested to degrade MRN [87] and could therefore influence other MRN-dependent functions.

(c) Summary

Similar to MCV, SV40 benefits from ATM and ATR activation, and DDR components are found at viral replication centres. In this way, the PIKK pathways interact with polyomaviruses and papillomaviruses similarly. However, unlike MCV, SV40 inhibits downstream checkpoint activation by targeting p53, similar to HPV. SV40 therefore selectively activates upstream PIKK signalling to promote viral replication while preventing downstream cell-cycle arrest.

7. Kaposi's sarcoma-associated herpesvirus

Kaposi's sarcoma-associated herpesvirus (KSHV) is a large, double-stranded DNA virus of the γ-herpesvirus family associated with Kaposi's sarcoma [92], primary effusion lymphoma (PEL), and multicentric Castleman's disease [93]. Like all herpesviruses, the KSHV life cycle has lytic and latent stages. During latency, viral proteins and RNA promote cell cycle progression and inhibit cell death. These factors include the latency-associated nuclear antigen (LANA), viral cyclin (v-Cyclin), viral FLICE inhibitory protein (v-FLIP) and KSHV-encoded miRNAs [93,94]. Upon various stress stimuli, KSHV reactivates from latency and produces mature virions [95,96]. Efficient viral DNA replication involves activation of the host DDR, and the lytic stage of the KSHV life cycle is most intertwined with the host PIKK family.

(a) Ataxia telangiectasia mutated, and ataxia telangiectasia and Rad3 related kinases

Similar to HPV, MCV and SV40, KSHV infection activates ATM and ATR, which promote productive viral replication [97]. A conserved herpesvirus kinase, ORF36, phosphorylates the ATM activator TIP60 [98], and MRN associates with viral replication centres [97]. However, ATM, CHK1 and downstream ATM substrates are inactivated later in the lytic reactivation cycle [99]. Inhibition of ATM or ATR activity results in decreased KSHV replication, and ATR promotion of viral replication is independent of downstream CHK1 [97]. Together, these findings demonstrate that the ATM and ATR are important for lytic replication.

While necessary for lytic reactivation and productive replication, the DDR inhibits establishment of latency. Overexpression of v-Cyclin arrests the cell cycle, leading to senescence where latency cannot be established [100]. This is concomitant with a strong increase in DDR markers [100]. ATM inhibition in cells overexpressing v-Cyclin relieves cell-cycle arrest [100]. In contrast, infection of primary human fibroblasts does not induce senescence [101]. While infected fibroblasts express high levels of v-Cyclin that drive transient hyper-proliferation and robust DDR activation, senescence is blocked by v-FLIP [101]. Thus, while latent gene expression activates the DDR, KSHV has evolved to block inhibition by the DDR via additional latency proteins.

(b) Summary

Like HPV and SV40, KSHV exploits the DDR to promote productive replication. While direct activation of cell division by latency proteins provides a potential link to oncogenesis [93], emerging evidence points to lytic reactivation as being critical in the initial events of cancer progression or establishment of a proper tumour microenvironment [102,103]. DDR activation impairs establishment of latency, thus KSHV blocks detrimental downstream effects. This dual-stage life cycle with positive and negative interactions with the DDR is a conserved feature among γ-herpesviruses.

8. Epstein–Barr virus

Epstein–Barr virus (EBV) is a γ-herpesvirus that infects nearly all adult humans worldwide [104] and establishes lifelong latency in memory B cells. EBV causes nearly all cases of endemic Burkitt lymphoma [105] and is implicated in diverse cancers of B cell and epithelial cell origin, including diffuse large B cell lymphoma, Hodgkin lymphoma, nasopharyngeal carcinoma and gastric cancer [104]. EBV drives cellular replication through multiple tightly regulated latency programmes. These include expression of the Epstein–Barr nuclear antigens (EBNAs) or latent membrane proteins (LMPs) [106]. These latency programmes are temporally regulated after infection, and are present in all EBV-induced cancers [107]. Like all herpesviruses, EBV can undergo lytic reactivation, a highly regulated process that requires viral proteins BZLF1 and BRLF1 [108]. BZLF1 and BRLF1 initiate immediate-early and early gene expression leading to productive viral replication.

While EBV-transformed cell lines are karyotypically stable [109], chromosomal abnormalities such as dicentric chromosomes and telomere dysfunction are detected in EBV-associated cancers [110]. EBNA1 is sufficient to induce chromosomal dysfunction and DDR upregulation [110,111]. Follow-up studies demonstrated telomere dysfunction in EBV-positive Burkitt lymphoma cells, while corresponding EBV-negative lines contained normal telomeres [112].

(a) Ataxia telangiectasia mutated, and ataxia telangiectasia and Rad3 related kinases

EBV regulates both ATM and ATR signalling, with consequences that depend on the stage of the virus life cycle. Early after infection, EBV expresses latent-cycle proteins that induce transient host cell hyper-proliferation and robust DDR activation with co-localization of γH2AX, CHK2 and 53BP1 at nuclear foci in the absence of productive viral replication [113]. These foci do not localize to viral DNA, suggesting they are at sites of damage in the cellular genome. Loss of the viral latency protein EBNA3C exacerbates the DDR [113], although the mechanism remains unknown. Furthermore, inhibition of ATM or CHK2 increases cellular proliferation leading to more efficient viral latency establishment [114]. In contrast, ATM activation seems to be necessary for efficient viral lytic reactivation. Expression of the immediate-early lytic gene BZLF1 promotes activation of ATM signalling and localization of ATM and MRN at EBV replication centres [115]. ATM activity is important for lytic reactivation in response to several stimulants in both B cells and epithelial cells [98,116]. ATM, ATR and TIP60 are also activated by EBV BGLF4, a homologue of KSHV ORF36 [98,117]. Downstream of ATR, EBV-induced STAT3 activity inhibits ATR-dependent CHK1 phosphorylation [118]. Thus, the effect of DDR signalling on EBV depends on the stage of the viral life cycle.

(b) Summary

Like KSHV, the life cycle of EBV is divided into two different stages of latency establishment/maintenance and lytic reactivation. Activation of the DDR appears to be crucial for efficient viral replication during lytic reactivation, and EBV encodes different lytic cycle proteins to achieve this goal. As with KSHV, the establishment of latency after primary infection is inhibited by DDR activation. To overcome this inhibition, EBV encodes EBNA3C to blunt the growth-suppressive DDR, implying that the DDR is a barrier to persistence that must be overcome.

9. Concluding remarks

To establish productive viral replication or long-term persistence, DNA tumour viruses manipulate nuclear processes, and manipulation of these processes can indirectly lead to cellular transformation. Here, we have discussed how DNA tumour viruses regulate the PIKK signalling cascades of the cellular DDR. The presence of virus genomic DNA or viral proteins can activate the DDR, and activation has a range of consequences for viral replication. Features of the DDR can present restrictive obstacles that are dismantled, while other activities can be harnessed to promote replication. The impact of DDR activation can also depend on the stage of the viral life cycle. Activation of DDR signalling pathways normally results in cell-cycle arrest, but since such arrest could suppress viral replication, all of the viruses discussed here inhibit checkpoint activation. This allows viruses to activate selectively the upstream DDR proteins or pathways that promote viral replication without suffering from the downstream effects on the cell cycle. While this benefits viral replication, it can have disastrous outcomes for host genome integrity, contributing to the oncogenic potential of these viruses. The intricate relationship between DNA tumour viruses and the DDR remains an exciting field with the potential to reveal fundamental regulatory mechanisms that maintain cellular genome stability and prevent cellular transformation. Interactions between viruses and the DDR could also present therapeutic opportunities to interrupt viral replication and prevent viral persistence and oncogenesis.

Data accessibility. This article has no additional data.

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