High-Risk Human Papillomaviruses and DNA Repair

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1 Introduction

Papillomaviruses (PVs) are non-enveloped DNA viruses that are the causative agents of benign and malignant epithelial lesions. In the 1930s, investigators found that filtered extracts of papillomas from cottontail rabbits could establish new infections in uninfected rabbits (Zhou et al. 2013; Lowy 2007). While some rabbits cleared these lesions, others developed squamous cell carcinomas. In the 1940s, Peyton Rous determined that treating cottontail rabbit papillomavirus lesions with coal tar resulted in the rapid appearance of carcinomas suggesting that other factors besides viral infection could influence progression (Zhou et al. 2013; Lowy 2007). Furthermore, extracts from papillomas of the mouth of rabbits were not able to produce lesions in genitalia indicating that these viruses exhibited tissue tropism. Subsequently, bovine papillomavirus 1 was found to induce large lesions in cows and became a main focus of study for decades due to the ease in harvesting virions from these lesions and BPV's ability to transform mouse fibroblasts (Lowy 2007; Kawai and Akira 2011). Around the turn of the century, it was observed that cutaneous warts from human hands were not transmissible to the genitalia; however, it was not until the seventies that it was determined that different papillomavirus types were responsible and exhibited tissue tropism. While some papillomavirus induced warts remained benign, others had the ability to progress to squamous cell carcinoma. These findings preceded the realization that cervical neoplasias not only resembled viral papillomas, but contained high amounts of human papillomavirus DNAs (HPV) (Zhou et al. 2013; Lowy 2007).

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Genital human papillomaviruses (HPVs) are spread primarily through sexual contact, and their life cycles are intimately linked to the differentiation of squamous epithelia. Over 75% of sexually active individuals have been infected with genital HPVs at some point in their lives (Pivarcsi et al. 2004; Castellsagué 2008). Over 200 types of HPVs have been identified and approximately 40 infect the genital tract. Of these, 12 are considered high-risk types and include HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. Low-risk HPV types (e.g., HPV 6 or 11) induce benign lesions such as genital warts and laryngeal papillomas, while high-risk types are the etiological agents of cervical cancer, anogenital cancers, as well as many head and neck squamous cell carcinomas (HNSCCs). High-risk types are responsible for over 7% of all cancers worldwide (Zhou et al. 2013; Lowy 2007; Forman et al. 2012), and over 99% of cases of cervical cancer are HPV-associated (Lowy 2007; Kawai and Akira 2011; Moody and Laimins 2008). In the US, half of the approximately 10,000 women diagnosed with cervical cancer each year will die from this disease (Pivarcsi et al. 2004; Castellsagué 2008; Parkin and Bray 2006). Recent studies suggest that high-risk HPVs are responsible for over 60% of oropharyngeal cancers, and this number has been increasing over the last decade (Chaturvedi 2012; Robinson et al. 2013; Schiffman et al. 2016). Three prophylactic HPV vaccines have been developed that target both high-risk and low-risk types. The vaccines consist of multivalent virus-like particles from specific high-risk and common low-risk HPV types. The bivalent version protects against HPV 16 and 18 infections, the quadrivalent version targets HPV 16, 18, 11, and 6, while the nanovalent version is directed against HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58. These vaccines are highly effective in blocking initial infection by HPV, but have no effect on pre-existing infections (Prue et al. 2017; Lu et al. 2011). There are no effective treatments outside of surgery or cryotherapy for treating existing HPV lesions, and therefore, it remains critical to understand the viral life cycle to uncover how viral infection progresses to malignancy and to identify new therapeutics. One of the host pathways that HPV hijacks is the DNA damage response (DDR) which is required for HPV's differentiation-dependent amplification. Before describing the DDR and its role in HPV infection, it is first important to discuss the multiple factors that regulate the viral life cycle.

2 Genome Organization

HPV genomes consist of double-stranded circular DNAs that are approximately 8 kB is size and encode for between 6 and 8 open-reading frames. Viral transcription takes place from a single DNA strand, and early gene expression is regulated in large part by alternative splicing (Fig. 1). Upon infection, viral genomes establish themselves as nuclear extrachromosomal elements or episomes, and maintenance of these elements is necessary for viral genome replication and persistence. A region 500–1000 base pairs upstream of the early coding sequences that is alternatively referred to as the upstream regulatory region (URR), the long

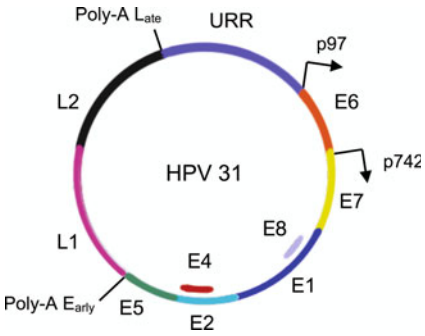

Fig. 1 Schematic representation of the HPV 31 genome. Circular map of HPV 31 genome identifying the upstream regulatory region (URR), which is a non-coding region that contains binding sites for multiple transcription factors and the origin of replication. The genome contains both an early (p97) and late promoter (p742), along with early and late polyadenylation sites. The late promoter is activated upon epithelial differentiation. The early genes include, E1, E2, E6, E7, E1^E4, E5, and E8^E2C. The two late capsid proteins are encoded by the L1 and L2 genes

control region (LCR), or the non-coding region (NCR), regulates early transcription and contains the viral origin of replication (McBride et al. 2012; Mighty and Laimins 2013). Early (E for early) transcription precedes productive viral replication and directs expression of polycistronic messages that encode for E1, E2, E1^E4, E5, E8^E2C, E6, and E7. The E1 and E2 proteins are DNA binding factors that bind to sequences in the URR and help to recruit cellular replication factors to viral origins. E2 also helps regulate early gene expression while E8^E2C acts primarily as a repressor. E6 and E7 are the two viral oncoproteins that control cell-cycle progression and allow for cells to remain active in the cell cycle upon differentiation to allow for genome amplification in suprabasal layers. E1^E4 and E5 regulate late viral events (Moody and Laimins 2008). Early viral transcripts are translated using a "leaky scanning," mechanism where the first open-reading frame is translated at a high rate while the sequential ORFs are translated at lower rates. This mechanism contributes to modulation of the levels of viral proteins, so that E6 and E7 are translated at high rates and others such as E5 are translated at very low levels (Remm et al. 1999). Upon differentiation, E1^E4, E5, L1, and L2 are expressed from the late viral promoter (p742) located in the E7 ORF (Moody and Laimins 2008; Beglin et al. 2009). Late viral genes L1 and L2 encode the major and minor capsid proteins of HPV and are critical for viral entry and egress. In many HPV-induced cancers, high-risk HPV genomes are found integrated into host DNAs. Integration leads to disruption of E2 expression which results in increased expression of the HPV encoded oncogenes E6 and E7.

Keratinocyte differentiation, HPV replication, and oncogenesis: The life cycle of human papillomavirus is closely linked to epithelial differentiation. Differentiated normal keratinocytes are divided into four distinct layers: the *stratum basale*, the *stratum spinosum*, the *stratum granulosum*, and the *stratum corneum*.

The basal layer is made up of cells that have not yet differentiated and remain proliferative, while the other three layers represent varying degrees of differentiation. Genital HPVs infect cells in the basal layer of the epithelium that become exposed through microabrasions generated through sexual activity (Fig. 2). Following entry, the viral genomes migrate to the nucleus where they associate with PML bodies which may help initiate viral transcription (McBride 2008). Within the nucleus, the virus rapidly undergoes several rounds of amplification using host replication machinery, reaching approximately 50 copies per cell. In persistently infected basal cells, viral genomes are replicated in synchrony with cellular replication and distributed equally to daughter cells at approximately 50–100 copies per cell. Evidence from two-dimensional gel electrophoresis suggests that genome replication during maintenance may occur through the formation of theta-structures (Flores and Lambert 1997). Cells in the basal layer can remain infected for years as they evade immune surveillance and provide a repository for production of new viruses. After cell division, one daughter cell moves away from the basal layer and begins the process of differentiation. Normal keratinocytes exit the cell cycle upon differentiation, but viral infection prevents cell-cycle exit, locking cells in G1 initially, but subsequently pushing cells to re-renter S/G2 where the genomes are amplified to approximately 1000 copies per cell. Coincident with amplification the capsid proteins, L1 and L2 are synthesized which self-assemble into icosahedrons and package viral DNA. Newly synthesized viruses are then released from cells in the *stratum corneum*.

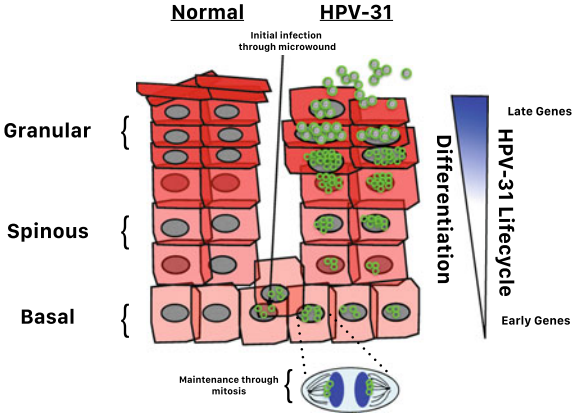

Fig. 2 HPV life cycle is intimately linked to epithelial differentiation. HPV infects cells in the basal layer that are exposed through microwounds and establishes its genomes at low copy number in these cells while expressing early genes. The HPV copy number in basal cells is maintained constant through cell division. Upon differentiation, one daughter cell moves away from the basal layer and begins differentiating leading to activation of the late promoter, amplification of the HPV genome, and subsequent assembly of progeny virions

Progression to malignancy often occurs in cells in which viral genomes have integrated into host chromosomes, and this leads to increased expression of the E6 and E7 oncoproteins. Little is known about what influences integration and how E6 and E7 contribute to this process though both can induce genetic instability in cells. In many cases of HPV-associated malignancies, HPV integrates at random fragile sites into the host genome retaining expression of only E6 and E7. Integration may contribute to genetic instability through the generation of double-stranded breaks and the activation of the ataxia telangiectasia mutated pathway (ATM) leading to mutations in the host genome (Kadaja et al. 2009).

3 Viral Oncoproteins

E6 and E7: The E6 and E7 proteins play major roles in manipulating the cell cycle in HPV-infected cells. Although present in low-risk HPV types, only high-risk E6 and E7 exhibit transformation activity. Although E6 and E7 are able individually to transform NIH-3T3 mouse fibroblasts, both are required for efficient immortalization of keratinocytes (Howley and Lowy 2007).

A major function of high-risk E7 is to bind and inhibit the activity of the retinoblastoma family of proteins, including pRb, p107, and p130. High-risk E7 proteins are approximately17 kDa in size and contain three conserved domains (CR1, CR2, and CR3) that share extensive homologies with adenovirus E1A (Zhang et al. 2006). High-risk E7 binds pRb ten-times more efficiently than the E7 of low-risk types (Münger et al. 1989). The CR1 and CR2 domains are both implicated in pRb binding and degradation with CR2 containing a conserved LXCXE motif. CR3 contains two zinc-finger domains that interact with the C-terminal domain of pRb and may play a role in stabilizing binding (Liu et al. 2006). E7 binds pRb and targets it for ubiquitin-mediated proteosomal degradation (Boyer et al. 1996). This degradation permits constitutive activation of the transcription factor E2F1 that regulates G1-S transition in both undifferentiated and differentiated cells (Moody and Laimins 2010). E2F normally associates with the Rb family members in G1, which represses its activation function and is released upon phosphorylation of Rb by CDK kinases in a cell-cycle dependent manner. E2F factors are bound to the promoters of genes that are normally expressed in S-phase, and release from Rb binding leads to activation of expression. p130 and p107 are also targets of E7 binding and act similar to Rb (Zhang et al. 2006). Interestingly, E7 also induces genetic instability by causing missegregation of chromosomes in a manner independent of its pRb binding through the degradation of the cyclin-dependent kinase inhibitor p21 as well as through alterations in centrosomes (Duensing and Münger 2003). In addition to its role in pRb family binding and degradation, E7 regulates E2Fs activity through its interactions with histone deacetylases (HDACS). HDACS remove acetyl groups from histones, and this results in heterochromatinization and repression of a DNA locus. E7 binds HDACS through the CR3, zinc-finger binding domain (Longworth and Laimins 2004).

As a by-product of altering cell-cycle progression, E7 expression leads to enhanced levels of p53 that can lead to apoptosis (Demers et al. 1994). To counteract the effects of elevated p53 levels, the high-risk E6 proteins have evolved to degrade p53. A major role of high-risk E6 in the HPV life cycle is to bind p53 and recruit it into a complex with the E3-ubiquitin ligase E6 associated protein (E6AP), which ubiquitinates p53, targeting it for proteosomal degradation. E6 can also bind the coactivators p300/CBP preventing p53 acetylation which also inhibits its transcription activation ability (Zimmermann et al. 1999; Gu et al. 1997). Another major target of p53 is p21, and E6 induced degradation of p53 prevents p21 mediated G1/S or G2/M checkpoint arrest, as well as apoptosis. This allows for HPV to replicate during an aberrant cell cycle. Recent observations demonstrate that HPV genomes containing knockout mutations in E6 are deficient in viral maintenance and knockdown of p53 restores episomal maintenance (Lorenz et al. 2013). Other studies have demonstrated that E6's role in viral maintenance and cell proliferation also relies on its PDZ binding motif (Lee and Laimins 2004; Nicolaides et al. 2011). It has been shown that E6 interacts with several PDZ domain containing proteins, including Dlg, MAGI-1/2/3, MUPP1, and Scribble all of which E6 targets for proteosomal degradation (Massimi et al. 2004). These proteins are thought to organize complexes of proteins that contribute to tumor suppressor activity. Another critical activity of E6 is the activation of htert, the catalytic subunit of telomerase. This is important for keratinocyte immortalization through direct interaction of E6 and E6AP with the htert promoter (Liu et al. 2009). Increased expression of either htert or c-myc independent of E6 expression readily duplicates E6's effects on htert, demonstrating its importance in immortalization (Galloway et al. 1998),(Liu et al. 2007). Recent studies have outlined a role for high-risk HPV E6 in regulating chromatin modifiers such as Tip60 and SET7 which inhibit transcriptional regulation by p53 indicating that E6 can control p53 expression at various stages (Vande Pol and Klingelhutz 2013). The high-risk E6 and E7 are sufficient to immortalize keratinocytes through the binding and degrading of p53 and pRb and the upregulation of telomerase activity. It is important to note, however, that in low-risk HPV types, immortalization does not occur due to E6 and E7's lower affinities for p53 and Rb, and E6's inability to activate htert expression (Van Doorslaer and Burk 2012).

E5: The HPV E5 protein is expressed in both early and late stages of the viral life cycle and associates with the Golgi apparatus, endoplasmic reticulum, as well as endosomal and nuclear membranes (Conrad et al. 1993; Hausen zur 2000; Ashrafi et al. 2005). Expression of E5 in mouse fibroblasts and human keratinocytes results in EGF-dependent proliferation suggesting that E5 may serve as a regulator of epidermal growth factor receptor (EGFR) activity, analogous to BPV E5's regulation of platelet-derived growth factor beta (PDGFR-β) that leads to transformation (Petti and Dimaio 1994; Straight et al. 1995). Transgenic mice expressing HPV 16 E5 under the control of the K14 promoter develop large tumors suggesting that E5 can act as an oncoprotein (Genther Williams et al. 2005). E5 also enhances the efficiency of E6 and E7 transformation but exhibits no transformation activity when expressed by itself (Valle and Banks 1995). Studies in COS cells demonstrate that

HPV 16 E5 is capable of interacting with a variety of transmembrane receptor proteins including EGFR and PDGFR-β. Furthermore, E5 is able to stabilize endosome acidification and cell surface EGFR expression, in turn stabilizing EGFR at endosomes upon stimulation with ligand (Straight et al. 1995; Hwang et al. 1995). EGFR stabilization may be associated with oncogenic activity due to enhanced EGFR-mediated mitogenic activity. Interestingly, HPV-31 genomes with E5 knockouts lose the ability to activate late viral transcription and amplification suggesting that E5 may contribute to regulation of late viral functions (Fehrmann et al. 2003; Genther et al. 2003). Recent studies indicate other targets for E5 including the ER-associated lipoprotein A4 that is linked to Akt-mediated proliferative capacity, and Bap-31 an ER-associated membrane complex shuttling protein, that among other functions shuttles MHC-I proteins through the ER. Both A4 and Bap31 are regulated by E5 leading to enhanced cellular proliferation (Regan and Laimins 2008; Halavaty et al. 2014).

4 The DNA Damage Response

The host DNA damage response (DDR) has evolved to repair single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) breaks as well as inter- and instra-strand cross-links induced by external damaging agents such as radiation or replication errors (Fradet-Turcotte et al. 2016). The DDR repairs thousands of lesions per cell per day and is vitally important for cell-cycle progression as well as to maintain genetic fidelity (Fradet-Turcotte et al. 2016; Wallace and Galloway 2014). If these lesions are not repaired, cells accumulate damage which leads to genetic instability that contributes to progression to malignancy. The PI3K-related protein kinases Ataxia-telengectasia mutated (ATM) and Ataxia-telengectasia Rad3 related (ATR), and DNA-dependent protein kinase (DNA-PK) are activated in response to different types of DNA breaks resulting in the activation of downstream signaling pathways. This activation leads to cell-cycle checkpoint arrest, repair, or apoptosis in the case of damage that cannot be repaired. ATR and ATM cross-talk as they share many downstream substrates while knockdown or mutation of either results in aberrant signaling and an impaired checkpoint response. Many cancers lack appropriate G1 checkpoint control and rely on ATR and ATM to fix DNA lesions that frequently arise. These pathways are also amplified in many cancers (Rundle et al. 2017).

The ATM pathway is activated in response to double-stranded breaks (DSBs) leading to the autophopshorylation of the ATM kinase at serine 1981. The autophosphorylation of ATM then recruits the MRN complex that is made up of Nijmegen Breakage Syndrome 1 (NBS1) Rad50 and Mre11 to the DSB. ATM also phosphorylates the modified histone H2AX at serine 139 known as ɣ-H2AX which bind to regions surrounding the lesion further amplifying the DDR. ɣ-H2AX in turn recruits mediator of DNA damage checkpoint protein 1 (MDC1) which is responsible for recruiting the ubiquitin ligases RNF8 and RNF168 to the DNA

lesion as well as the non-homologous end-joining (NHEJ) and homologous recombination (HR) repair factors such as 53BP1 and BRCA1 to the lesion (Uckelmann and Sixma 2017; Mattiroli et al. 2012; Doil et al. 2009). 53BP1 and BRCA1 recruitment mediates the choice between which of the two repair pathways fixes the lesion (Fradet-Turcotte et al. 2016). NHEJ repair is the major repair mechanism in mammalian cells and occurs without extensive processing and the presence of the sister chromatid. p53BP1 is recruited to the ends of DSBs along with the Ku70-Ku80 complex that tethers the ends together (Fradet-Turcotte et al. 2016; Chapman et al. 2012). The ends are then ligated in an error-prone manner by DNA ligase-IV and X-ray repair cross-complementing protein 4 (XRCC4) (Chapman et al. 2012; Burma and Chen 2004). This process occurs primarily in the G1 phase.

Homologous recombination occurs in S/G2 phases as it requires the presence of the sister chromatid for homology-based repair and strand invasion. In S/G2 phases BRCA1, CtBP-interacting protein (CtIP) and the MRN complex are recruited to the DSB following ATM activation to start DNA end resection which is a necessary step for HR. 5′–3′ end resection by the exonuclease Exo1, Dna2 nuclease, and the helicase Sgs1 are required for the resection and establishment of ssDNA. This is followed by the recruitment of HR factors and sister strand invasion (Niu et al. 2010). The ssDNA is then coated with the ssDNA stabilizing heterotrimeric replication protein A (RPA). BRCA1 recruitment to the DSB promotes recruitment of BRCA2 through the BRCA1-BRCA2 partner and localizer of BRCA2 (PALB2) (Rohini Roy JCSNP 2012). This is followed by displacement of RPA from the ssDNA and recruitment of Rad51 which results in its polymerization and formation of Rad51 nucleofilaments that search for sister chromatid homology leading to repair. If the damage is irreparable, apoptosis is induced through the action of BRCA1 (Fradet-Turcotte et al. 2016; Yuan et al. 1999; Chen et al. 1998).

Although ATM has a major role in DNA repair, it also plays a role in regulating cell-cycle checkpoints in efforts to maintain integrity. Cell-cycle checkpoints provide cells with a means to delay replication to allow for repair or apoptosis and are regulated by an extensive signaling cascade. ATM's role in cell-cycle checkpoint control was first realized when it was found that ATM was required for p53 activation. p53 is the classical tumor suppressor that is important for regulating the G1/S-phase checkpoint (Derheimer and Kastan 2010). Upon DNA damage, activated ATM phosphorylates p53, mouse double minute 2 homolog (MDM2), as well as checkpoint Kinase 2 (CHK2) leading to activation of the kinase inhibitor p21. This results in the inhibition of cyclin-E/CDK complexes and halts the G1/S transition. ATM signaling also plays roles in S-phase and intra-S-phase arrest by activating several proteins including structural maintenance of chromosomes protein 1 (SMC-1), NBS1, Fanconi anemia group D2 protein (FANCD2), and CHK2.

ATR responds to ssDNA breaks induced by radiation, genotoxic stress, depleted nucleotide pools, as well as replication stress due to stalled replication forks (Cimprich and Cortez 2008; Saldivar et al. 2017). ATR is recruited to ssDNA, i.e., that has been recognized by RPA with its partner ATR-interacting protein (ATRIP) which contains an RPA binding domain. This also leads to the further recruitment

of the proliferating cell nuclear antigen (PCNA) like Rad9-Rad1-Hus1 (9-1-1) complex that loads onto junctional dsDNA near the RPA-coated DNAs. The 9-1-1-complex recruits DNA topoisomerase 2-binding protein 1 (TOPBP1) which interacts with ATR and regulates its activation.

ATR activation leads to the phosphorylation of checkpoint protein 1 (CHK1) at two different sites, which is important for the regulation of cell-cycle checkpoints. This phosphorylation is mediated by Claspin which interacts with Rad17 part of the 9-1-1 clamp (Cimprich and Cortez 2008). Activation of CHK1 leads to dissociation from chromatin as well as signaling to the CDC25 phosphatases which prevent cell entry into mitosis (Smits et al. 2006). ATR also plays a role in coordinating origin firing and stabilizing stalled replication forks making it essential for cell viability even from very early embryonic stages (Shechter et al. 2004).

5 The DNA Damage Response and HPV

High-risk HPVs constitutively activate both the ATM and ATR DNA damage repair pathways, and this is necessary for productive replication in differentiating cells. The HPV E7 and E1 proteins can independently activate the ATM and ATR pathways. The E7 protein acts through the innate immune regulator STAT-5 which in turn activates ATM through its effects on the acetyltranferase Tip60. At the same time STAT-5 activates ATR through increased expression of the TopBP1 protein. Tip60 must acetylate ATM prior to its activation by phosphorylation and TopBP1 forms complexes with ATR resulting in its phosphorylation and recruitment to sites of DNA breaks. One by-product of this activation is the induction of genetic instability and as well as viral genome integration which may contribute to the development of virally induced cancers.

High-risk HPVs activate ATM and ATR pathways in both undifferentiated and differentiated cells. Importantly, small molecule inhibitors of either ATM or ATR prevent the differentiation-dependent amplification of HPV (Moody and Laimins 2009; Hong et al. 2015; Edwards et al. 2013). Inhibition of ATM or CHK2 prevents HPV amplification, and this results in reduced levels of Cdc25c which in turn regulates transition into G2, which is when HPV genome amplification occurs (Moody and Laimins 2009). HPV genomes are recruited to nuclear replication centers that contain many members of the DDR including, TopBP1, pATM, γ-H2AX, pCHK1, pCHK2, NBS1, BRCA1, pATR, Rad51, and FANCD2 (Kadaja et al. 2009; Wallace and Galloway 2014; Satsuka et al. 2015; Spriggs and Laimins 2017). In addition, shRNA knockdown studies have shown that Rad51, BRCA1, and NBS1 are required for differentiation-dependent amplification (Anacker et al. 2014). Interestingly, FANCD2, ATR and TopBP1, NBS1, and SMC1 are also required for efficient viral maintenance in undifferentiated cells (Hong et al. 2015; Spriggs and Laimins 2017; Anacker et al. 2014; Mehta et al. 2015). Many of these factors have been shown to bind to HPV genomes using ChIP analyses (Anacker et al. 2014; Gillespie et al. 2012).

Constitutive activation of the cell cycle by E6 and E7 leads to the depletion of free nucleotide pools, replication stress, and genomic instability due to the multiple origin firing and stalled replication forks (Bester et al. 2011). Recent studies indicate that high-risk E6 and E7 induce DNA breaks in both viral and cellular DNAs. Interestingly, homologous recombination repair factors are then preferentially recruited to viral DNAs to repair the breaks (Mehta and Laimins 2018). This preferential recruitment of DDR factors such as RAD51 and BRCA1 results in rapid repair of breaks and genome amplification that occurs in G2. This is a novel mechanism that links viral induction of DNA breaks to amplification of viral genomes as well as genetic instability in host chromsomes.

6 Summary

Human papillomaviruses are the causative agents of many anogenital cancers including almost all cervical cancers. In addition, over 60% of oropharyngeal cancers are associated with infection by high-risk HPVs, and the numbers are increasing rapidly in Western countries. While prophylactic vaccines have been developed that are highly effective in blocking initial infections, they have no effect

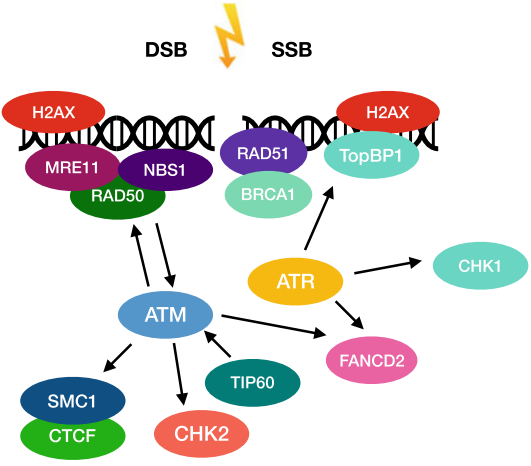

Fig. 3 Schematic of the DNA damage response pathway factors demonstrated to play a role in the HPV life cycle. The ATM pathway is activated in response to double-stranded breaks while ATR responds to single-strand DNA breaks. Activation of these pathways leads to checkpoint signaling, repair, and/or apoptosis. HPV nuclear replication foci contain members of these pathways including TopBP1, pATM, H2AX, pCHK1, CHK2, NBS1, BRCA1, ATR, Rad51, and FANCD2. Homologous recombination repair factors, Rad51, BRCA1, and NBS1 are all required for differentiation-dependent amplification of HPV. FANCD2, ATR, TopBP1, NBS1, and SMC1 are required for efficient maintenance of viral episomes in undifferentiated cells

on existing virally caused lesions. The life cycle of high-risk HPVs is dependent upon differentiation and activation of host DNA repair pathways. This includes both ATM and ATR pathways whose activation is required for both productive replication as well as stable maintenance of episomes. HPV proteins activate the DNA damage repair pathways through induction of DNA breaks in both cellular and viral DNAs. The preferential repair of breaks in viral genomes results in productive replication of HPV DNAs while at the same time promoting genetic instability in host chromosomes (Fig. 3).

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