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Ubiquitination at the interface of tumor viruses and DNA damage responses

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Abstract

Viruses exploit cellular ubiquitination machinery to shape the host proteome and promote productive infection. Among the cellular processes influenced by viral manipulation of ubiquitination is the DNA damage response, a network of cellular signaling pathways that sense and respond to genomic damage. This host-pathogen interaction is particularly important during virus replication and transformation by DNA tumor viruses. Manipulating DDR pathways can promote virus replication but also impacts host genomic instability, potentially leading to cellular transformation and tumor formation. We review ways in which viruses are known to hijack the cellular ubiquitin system to reshape host DDR pathways.

Introduction

Viruses have evolved ways to manipulate the host proteome in order to evade antagonistic antiviral host responses and to generate cellular environments conducive to infection. One strategy employed by viruses is to harness cellular ubiquitin machinery to direct ubiquitin modification of cellular proteins [1–3]. Ubiquitination is a protein post-translational modification that either directs substrates toward proteasomal degradation or mediates non-degradative outcomes such as controlling protein interactions, location or function [4–6]. Dynamic regulation of ubiquitination by ubiquitin ligase and deubiquitinase (DUB) enzymes allows ubiquitin to act as a molecular switch for rapid cellular responses. Viruses redirect ubiquitination of target substrates through four main approaches: (1) viral-encoded E3

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ligases, (2) integration into cellular cullin E3 ligase complexes, (3) manipulation of cellular E3 ligases or (4) control of deubiquitinase activity (Figure 1).

Entry and amplification of exogenous viral DNA activates components of the host DNA damage response (DDR) signaling network, a cellular system integral to maintaining genomic stability by sensing and responding to compromised DNA [7,8]. In response to activation of DDR pathways, viruses manipulate these processes to promote productive infection [2,9–11]. Viral manipulation of cellular DDR pathways can also affect cellular transformation and oncogenesis [11]. The human “tumor viruses” include Human Papilloma Virus (HPV), Merkel Cell Polyomavirus (MCV), Kaposi’s Sarcoma-associated Herpes Virus (KSHV), Epstein-Barr Virus (EBV), Human T-Lymphotropic Virus 1 (HTLV-1) and Hepatitis B and C Viruses (HBV/HCV). Additionally, Adenovirus (AdV) and Simian Virus 40 (SV40) Polyomavirus can transform cells *in vitro* [12,13] although they are not known to cause human cancers. Human tumor virus infections are associated with over 20 different types of cancers and approximately 10% of diagnosed cancer cases worldwide [14].

Ubiquitination plays a critical role at the interface between tumor viruses and host DDR pathways. Ubiquitin is an integral component of DNA damage recognition and repair, exhibiting important functions in protein recruitment and removal at sites of DNA damage, as well as regulation of downstream effectors [15–18]. Viruses direct ubiquitination to affect cellular processes and facilitate viral DNA replication and progeny production [1–3]. This review highlights specific examples of ways that tumor viruses hijack host ubiquitin machinery to promote viral replication and manipulate host DDR responses. Finally, viral mediated ubiquitination that does not result in substrate degradation by the proteasome (non-degradative ubiquitination) is proposed as a potential strategy to alter the DDR.

Ubiquitin as a post-translation modification

Ubiquitination is a protein post-translational process whereby a ubiquitin molecule is covalently attached to a lysine residue of a protein substrate through a multi-step enzymatic reaction. Ubiquitin is first activated by an E1 ubiquitin activating enzyme. Next, the activated ubiquitin molecule is transferred to an E2 ubiquitin conjugating enzyme. Finally, ubiquitin is covalently attached to the protein substrate in a reaction catalyzed by an E3 ubiquitin ligase enzyme. The process of ubiquitination is highly dynamic and can be reversed by DUBs that catalyze removal or editing of ubiquitin on substrates. Ubiquitin molecules have seven lysines that can be ubiquitinated, which confers the ability to form polyubiquitin chains on protein substrates. The chain type, as defined by the amino acid position of the modified ubiquitin lysine, is thought to dictate the functional outcome of ubiquitination.

The most well-understood outcome of ubiquitination is proteasome-mediated degradation of substrates where polyubiquitin chains linked by lysine at position 48 of the ubiquitin molecule (K48-chains) direct substrates to the 26S proteasome [5]. The importance of non-degradative ubiquitination is becoming increasingly appreciated. Monoubiquitination, multi-monoubiquitination and non-degradative polyubiquitin chains (K6, K11, K27, K29, K33, K63) can regulate protein-protein interactions, alter protein functions or direct protein localization [4–6].

The DNA damage response preserves genome integrity

The cellular genome is under constant threat of damage by endogenous events and exogenous sources. The DDR signaling network is responsible for recognition and repair of DNA damage, driving the cell toward cell-cycle arrest, senescence, or DNA repair [7,8]. DDR proteins are broadly categorized into “sensors” of DNA damage, “transducers” of DDR signaling and downstream “effectors” of DNA repair and cell cycle control (Figure 2). The DDR network is mediated by three PIKKs (phosphatidylinositol 3-kinase-like protein kinase); ATM (ataxia telangiectasia mutated), ATR (ataxia telangiectasia and Rad3 related) and DNA-PK (DNA-dependent protein kinase) [19]. The MRN complex (MRE11, RAD50 and NBS1 proteins) senses double-strand breaks (DSBs) and activates ATM [20] through TIP60 [21]. ATM phosphorylates substrates involved in DNA repair (e.g. BRCA1 and RAD51), as well as cell cycle control and apoptosis (e.g. CHK2 and p53). DSBs are also sensed by the Ku70/Ku80 heterodimer, which activates DNA-PK, and leads to end-joining by DNA ligase IV [22]. The RPA protein coats single-strand DNA and recruits ATR through ATRIP (ATR-interacting protein) [23] and the 9–1–1 complex (RAD9, HUS1, RAD1), which binds the ATR-activating protein TOPBP1 [24]. ATR substrates control cell cycle and apoptosis (e.g. CHK1 and p53). Overall, there are hundreds of proteins associated with pathways mediating the DDR [25,26], and this extensive network of proteins and interactions provide an array of opportunities for viral manipulation.

Post-translational modifications coordinate complex cellular responses to DNA damage. Ubiquitin plays an integral role in orchestrating and regulating both assembly and disassembly of repair proteins at DNA damage sites [15–18]. Ubiquitination of many cellular proteins increases upon DNA damage [26]. K63-linked ubiquitination plays important roles in signaling for recruitment of DDR-associated proteins to DSBs, while protein turnover through K48-linked modification regulates levels of DDR proteins. Overall, there is a complex landscape of ubiquitination at DNA lesions that collectively serve to remodel chromatin and direct repair. In addition to roles at damage sites, ubiquitin functions to modulate downstream effectors of the DDR network that lead to cell cycle arrest and apoptosis.

Impacts of DDR signaling on viral infection

DDR signaling pathways can be induced as host cells react to viral genomes and/or by viral manipulation of DDR components. Activation of cellular DDR signaling has outcomes that are pro-viral or anti-viral. Signaling through tumor suppressors such as p53 leads to activation of cell cycle checkpoints that must be overcome by viruses. Processing of viral genomes by host DNA repair factors can limit virus replication. In contrast, many viruses utilize subsets of DNA repair factors during viral genome replication. Many previous reviews provide detailed descriptions of interactions between viruses and the DDR, especially viral oncoproteins that control cell cycle checkpoints [9–11,27–29].

Viruses harness ubiquitination to manipulate the host proteome

There are multiple ways in which viruses hijack host ubiquitin machinery (Figure 1). The mechanisms employed by viruses to harness cellular ubiquitination include: encoding viral proteins with E3 ligase activity (Figure 1, I), integrating into cellular protein complexes to

form host-viral cullin E3 ligases (Figure 1, II) or redirecting cellular E3 ligase activity (Figure 1, III). Viruses also exert control by removing ubiquitin through viral or cellular DUBs (Figure 1, IV). All these strategies are employed by viruses to modify key regulators of the cellular DDR machinery (Figure 2).

Viral proteins with E3 ligase activity alter DDR pathways

Components of the DDR network can be directly targeted by viral-encoded E3 ligases (Figure 1, I). One strategy is to manipulate recruitment of DNA repair proteins by targeting key steps in ubiquitination. RNF8-mediated histone ubiquitination is one of the first steps in DDR responses to DSBs [30–32]. ATM-dependent phosphorylation of MDC1 prompts E3 ligase RNF8 recruitment to breaks. RNF8 mediates K63-linked ubiquitination of H1-linker histones, which promotes recruitment of a second ligase RNF168, which ubiquitinates histone H2A and other cellular factors to generate assemblies of repair proteins. The HSV ICP0 protein is a RING-type E3 ligase that targets RNF8 and RNF168 for ubiquitination and degradation, to prevent localization of DDR factors at sites of incoming viral genomes [33,34]. ICP0 also ubiquitinates and degrades DNA-PK [35,36], which may influence circularization of viral genomes [37]. ICP0 targeting RNF8 and RNF168 is an intriguing example of viral-mediated ubiquitination used to directly target and suppress cellular ubiquitination. The DDR-associated E3 ligase RNF8 is also harnessed by the HTLV-1 protein Tax to redirect ubiquitination and suppress DDR responses and activate IKK/NF- κ B pathways [38].

Viral proteins integrate into cellular cullin complexes to target DDR pathways

Since E3 ubiquitin ligases confer substrate specificity, viruses predominantly interface with this step of the ubiquitin conjugation process. Cullin proteins act as scaffolds that interact with E2 binding proteins and substrate adaptors in cullin-RING E3 ligases (CRLs), which constitute the largest known family of E3 ligases [39]. Viruses commonly integrate into CRL complexes to act as substrate adaptors/receptors to redirect ubiquitination specificity (Figure 1, II).

The Ad5 E1B55K and E4orf6 proteins complex with cellular Cullin5 to form a CRL complex [40] that redirects ubiquitination to multiple DDR substrates. The Ad5 serotype E1B55K/E4orf6 CRL induces degradation of the MRN complex [41] and TIP60 [42] and prevents ATM activation [43]. The E1B55K/E4orf6 complex also promotes degradation of DNA repair proteins such as the BLM helicase [44] and DNA ligase IV [45]. Additionally, the Ad12 serotype degrades TOPBP1 to prevent ATR activation [46]. In the absence of viral proteins that target these repair proteins, AdV infection results in activation of DDR signaling, MRN inhibits viral replication, and viral genomes are joined into concatemers [41,47–50]. The MRN complex is also targeted by the SV40 large T antigen which binds Cullin7 to prevent ATM activation [51].

Downstream of DDR signaling, tumor suppressor p53 is phosphorylated to regulate cell cycle checkpoints and apoptosis in response to DNA damage. Phosphorylation results in p53 stabilization by preventing association with the MDM2 E3 ubiquitin ligase, thereby suppressing ubiquitin-mediated degradation. Many viruses counter p53 and other tumor

suppressors by inducing degradation, which ultimately contributes to genomic instability, cellular transformation and oncogenesis [52]. For example, the E1B55K/E4orf6 CRL complex ubiquitinates and degrades p53 [40]. The KSHV LANA protein also harnesses the same cellular Cullin5-complex, via a SOCS-box-like motif on the viral protein, and degrades p53 as well as the tumor suppressor von Hippel-Lindau [53]. The HPV E7 protein complexes with a Cullin2 CRL ligase to degrade Rb [54,55]. EBV protein BZLF1 functions as a substrate receptor in a Cullin2/5 complex to degrade p53 [56,57], and the EBNA3C viral protein forms a complex with SCF^{Skp2} to degrade Rb [58].

A recently published example of manipulating genome maintenance pathways comes from HBV where the HBx protein complexes with Cullin4-DDB1-ROC1 to form a CRL E3 ligase [59] that degrades DDR-associated proteins SMC5/6 [60,61], which promotes disassociation from viral episomal DNA and facilitates viral gene expression. It was shown that SMC5/6 knockdown increases HBV replication during infection with HBx deficient virus but had negligible effects during wildtype infection, indicating that SMC5/6 is targeted because it acts as a restriction factor against HBV.

Viral proteins regulate cellular E3 ligases that maintain genome integrity

Viral proteins can indirectly mediate ubiquitination by interacting with cellular E3 ligases (Figure 1, III). A quintessential example is the HPV E6 oncoprotein, which interacts with cellular E3 ligase E6AP (E6 associated protein) to redirect ubiquitination and degradation towards cellular substrates such as p53 [62–64]. HPV E6 also facilitates ubiquitin-mediated degradation of the DDR protein TIP60 through cellular E3 ligase EDD1/UBR5 [65] although the exact mechanism is unclear. The HPV E7 oncogene promotes proteasome-dependent degradation of tumor suppressor PTPN14 through the cellular E3 ligase UBR4 [66]. The Rb protein is degraded during HCV infection through an interaction of HCV NS5B with E6AP [67]. An alternative viral strategy is to suppress ubiquitination by cellular E3 ligases. For example, EBV LMP1 protein both disrupts interaction of MDM2 with p53 to suppress K48-polyubiquitin-mediated degradation, and additionally induces K63-polyubiquitin by TRAF2 to stabilize p53 [68]. Similarly, MCV viral sT protein inhibits Fbw7 of the SCF^{Fbw7} E3 ligase by binding via the LT-stabilization domain, thereby stabilizing SCF^{Fbw7} substrates such as LT, c-myc, cyclin E, PLK1 and YAP [69,70]. The combination of these strategies, which target tumor suppressors and genome guardians, facilitate viral propagation while also contributing to host genome instability during tumorigenesis.

Viral strategies to remove ubiquitin within the DDR

Deubiquitination can be employed by viruses to modify ubiquitin signaling pathways by either blocking or redirecting cellular DUBs, or through virally-encoded DUBs (Figure 1, IV). In response to DNA damage, PCNA (proliferating cell nuclear antigen) is monoubiquitinated by RAD18 to activate the translesion synthesis pathway of post-replication repair. PCNA accumulates at viral replication compartments of many DNA viruses, although its function in viral replication is unclear. The EBV late protein BPLF1 is a DUB that deubiquitinates PCNA, preventing recruitment of cellular proteins and disrupting DNA repair [71]. BPLF1 can also promote viral replication by acting as a deneddylase to

modify the activity of CRLs [72], which induces accumulation of the cellular replication licensing factor CDT1 and deregulates S-phase DNA synthesis. The BPLF1 protein may therefore have dual functions during infection which inhibit cellular DNA replication/repair and enhance viral DNA replication.

The herpesvirus-associated ubiquitin-specific protease (HAUSP), also known as USP7, was originally discovered through its interaction with the ICP0 E3 ligase of HSV-1 [73] and was subsequently implicated in several cellular processes including oncogenesis, tumor suppression, and epigenetic modifications [74,75]. The first substrate identified for USP7/HAUSP deubiquitination was tumor suppressor p53 whose half-life is extended by the DUB activity [76]. In addition to p53, its own regulator MDM2 is also a substrate for USP7/HAUSP which protects it from auto-ubiquitination [77]. USP7/HAUSP also appears to have direct roles in cellular DNA replication and DNA damage. Among its substrates are CHK1, CLASPIN and TIP60. USP7/HAUSP can regulate stability of cellular E3 ligases including RNF168 [78] and RNF169 [79] which have roles in ubiquitin-dependent DDR signaling. Several viral proteins interact with USP7/HAUSP, including EBNA1 of EBV [80], LANA of KSHV [81] and E1B55K of AdV [82]. In the case AdV, USP7 is required for efficient viral growth and cellular transformation by E1A/E1B55K oncoproteins [82]. USP7 also affects stability of E1B55K but there is no direct evidence that it is a substrate for deubiquitination. In addition to ICP0, other herpesvirus proteins bind USP7/HAUSP. The EBNA1 protein of EBV competes with p53 and MDM2 for the same binding pocket in USP7/HAUSP and thus lead to p53 instability [83,84]. In this way EBNA1 may modulate p53 in EBV-infected cells to promote survival by decreasing DNA damage-induced apoptosis [84]. There are likely to be other DUBs that are harnessed in similar ways to USP7 to regulate cellular processes such as DDR signaling.

Can viruses direct non-degradative ubiquitination?

The best-characterized function of ubiquitin is to direct substrate degradation, and this is also the most well-understood function of ubiquitination during host-pathogen interactions. However, ubiquitination that has non-degradative outcomes is clearly widespread and is likely to be similarly targeted and harnessed by viruses. There are currently very limited known examples of viruses directing non-degradative ubiquitin, although a few viral ligases have been shown to harness K63 ubiquitin linkages to counter host responses. These include EBV protein LMP1, which was shown to associate with K63-polyubiquitination of RIPK1/3 to suppress necroptosis, although did not directly ubiquitinate the substrate [85]. KSHV encodes K3 and K5, which demonstrate E3 ligase activity and are able to employ K63 poly-ubiquitin linkages to promote internalization and endosomal degradation of surface MHC molecules [86].

Harnessing non-degradative ubiquitination would vastly increase the potential for viruses to modulate the cellular proteome and could avoid collateral effects of inducing degradation of cellular regulators. Viral-mediated non-degradative ubiquitination could be employed to recruit cellular proteins onto viral genomes, to sequester antiviral functions, to modulate cellular signaling pathways or to control protein complex formation. There is an emerging body of literature suggesting that CRLs, which are generally known to direct degradative

ubiquitination, can in fact promote K63-linked non-degradative ubiquitination [87,88]. Ubiquitin linkages other than K48 could be conjugated by viral proteins that integrate into CRLs. It would also be expected that viral-encoded E3 ligases have evolved the ability to interact with appropriate cellular E2 enzymes to form non-degradative poly-ubiquitin linkages.

Non-degradative ubiquitination has been shown to be particularly relevant within the DDR [15–18] and could therefore be employed during viral manipulation. It was recently shown that FBXW7, which forms the CRL SCF^{FBXW7}, is a substrate of ATM and is recruited to sites of DNA damage. SCF^{FBXW7} interacts with XRCC4 and promotes K63-linked non-degradative polyubiquitination, which ultimately facilitates activation of NHEJ [87]. This example illustrates CRL-mediated non-degradative ubiquitination within the DDR and could be broadly exploited by viruses that recruit CRLs (Figure 1, II).

Despite the preponderance of examples of viral-mediated degradative ubiquitin, there is a relative dearth of known non-degradative events. This likely results from limited availability of experimental approaches but is poised to change. Advanced proteomics technologies now facilitate identification of non-degradative ubiquitination events. Whole cell proteomics and diglycine remnant profiling [89,90] allow global investigation of modifications by ubiquitin and ubiquitin-like proteins and resulting impacts on protein abundance. Bioinformatic analysis and integration of relevant datasets can generate a comprehensive view of the ubiquitin landscape and effects of ubiquitination on the proteome. Thus, classification of both degradative and non-degradative ubiquitination will likely drive future studies of ubiquitin in host-pathogen interactions, especially within DDR signaling pathways.

Conclusions

Cellular E3 ligases and DUBs are regulated dynamically in supramolecular complexes. These molecular machines are often manipulated by viruses to tip the balance in favor of viral processes and disruption of antiviral defenses. In some cases, modulation may be subtle enough to retain cellular functions, while in other cases, host processes may be completely disrupted or redirected. In this review we focused specifically on ubiquitin modifications but ubiquitin-like proteins are important in both DDR signaling and virus-host interactions. Another area that has not been extensively investigated is ubiquitin binding domains in viral proteins, although it is likely that viral proteins will exploit the power of these “readers” to recognize specific ubiquitin chain linkages.

Viruses have evolved a variety of strategies to harness host ubiquitin machinery to redirect ubiquitination of cellular substrates and shape the host proteome (Figure 1). Manipulating the DDR pathways, either by stimulating or blunting DDR responses, can ultimately promote productive viral infection. Furthermore, viral modulation of DDR responses likely contributes to cellular transformation and oncogenesis. Ubiquitin-mediated processes represent targets for antiviral therapeutics, with both E3 ligases and DUBs providing compelling targets for small molecule inhibitors. Understanding how viruses use ubiquitin to target the DDR could offer insights into therapeutic means to control virus infection and combat viral-mediated oncogenesis.

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References

1. Isaacson MK, Ploegh HL: Ubiquitination, ubiquitin-like modifiers, and deubiquitination in viral infection. *Cell Host Microbe* 2009, 5:559–570. [PubMed: 19527883]
2. Weitzman MD, Lilley CE, Chaurushiya MS: Changing the ubiquitin landscape during viral manipulation of the DNA damage response. *FEBS Lett* 2011, 585:2897–2906. [PubMed: 21549706]
3. Luo H: Interplay between the virus and the ubiquitin-proteasome system: molecular mechanism of viral pathogenesis. *Curr Opin Virol* 2016, 17:1–10. [PubMed: 26426962]
4. Yau R, Rape M: The increasing complexity of the ubiquitin code. *Nat Cell Biol* 2016, 18:579–586. [PubMed: 27230526]
5. Komander D, Rape M: The ubiquitin code. *Annu Rev Biochem* 2012, 81:203–229. [PubMed: 22524316]
6. Kulathu Y, Komander D: Atypical ubiquitylation - the unexplored world of polyubiquitin beyond Lys48 and Lys63 linkages. *Nat Rev Mol Cell Biol* 2012, 13:508–523. [PubMed: 22820888]
7. Jackson SP, Bartek J: The DNA-damage response in human biology and disease. *Nature* 2009, 461:1071–1078. [PubMed: 19847258]
8. Ciccia A, Elledge SJ: The DNA damage response: making it safe to play with knives. *Mol Cell* 2010, 40:179–204. [PubMed: 20965415]
9. Luftig MA: Viruses and the DNA Damage Response: Activation and Antagonism. *Annu Rev Virol* 2014, 1:605–625. [PubMed: 26958736]
10. Pancholi NJ, Price AM, Weitzman MD: Take your PIKK: tumour viruses and DNA damage response pathways. *Philos Trans R Soc Lond B Biol Sci* 2017, 372.
11. Hollingworth R, Grand RJ: Modulation of DNA damage and repair pathways by human tumour viruses. *Viruses* 2015, 7:2542–2591. [PubMed: 26008701]
12. Ender C, Dobner T: Cell transformation by human adenoviruses. *Curr Top Microbiol Immunol* 2004, 273:163–214. [PubMed: 14674602]
13. Pipas JM: SV40: Cell transformation and tumorigenesis. *Virology* 2009, 384:294–303. [PubMed: 19070883]
14. Lunn RM, Jahnke GD, Rabkin CS: Tumour virus epidemiology. *Philos Trans R Soc Lond B Biol Sci* 2017, 372.
15. Schwertman P, Bekker-Jensen S, Mailand N: Regulation of DNA double-strand break repair by ubiquitin and ubiquitin-like modifiers. *Nat Rev Mol Cell Biol* 2016, 17:379–394. [PubMed: 27211488]
16. Brinkmann K, Schell M, Hoppe T, Kashkar H: Regulation of the DNA damage response by ubiquitin conjugation. *Front Genet* 2015, 6:98. [PubMed: 25806049]
17. Brown JS, Jackson SP: Ubiquitylation, neddylation and the DNA damage response. *Open Biol* 2015, 5:150018. [PubMed: 25833379]
18. Jackson SP, Durocher D: Regulation of DNA damage responses by ubiquitin and SUMO. *Mol Cell* 2013, 49:795–807. [PubMed: 23416108]
19. Blackford AN, Jackson SP: ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response. *Mol Cell* 2017, 66:801–817. [PubMed: 28622525]
20. Lee JH, Paull TT: ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. *Science* 2005, 308:551–554. [PubMed: 15790808]
21. Sun Y, Jiang X, Chen S, Fernandes N, Price BD: A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. *Proc Natl Acad Sci U S A* 2005, 102:13182–13187. [PubMed: 16141325]

22. Nick McElhinny SA, Snowden CM, McCarville J, Ramsden DA: Ku recruits the XRCC4-ligase IV complex to DNA ends. *Mol Cell Biol* 2000, 20:2996–3003. [PubMed: 10757784]
23. Zou L, Elledge SJ: Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* 2003, 300:1542–1548. [PubMed: 12791985]
24. Delacroix S, Wagner JM, Kobayashi M, Yamamoto K, Karnitz LM: The Rad9-Hus1-Rad1 (9–1–1) clamp activates checkpoint signaling via TopBP1. *Genes Dev* 2007, 21:1472–1477. [PubMed: 17575048]
25. Pearl LH, Schierz AC, Ward SE, Al-Lazikani B, Pearl FM: Therapeutic opportunities within the DNA damage response. *Nat Rev Cancer* 2015, 15:166–180. [PubMed: 25709118]
26. Elia AE, Boardman AP, Wang DC, Huttlin EL, Everley RA, Dephoure N, Zhou C, Koren I, Gygi SP, Elledge SJ: Quantitative proteomic atlas of ubiquitination and acetylation in the DNA damage response. *Molecular cell* 2015, 59:867–881.* This study uses global proteomics to identify ubiquitin and acetylation dynamics during DNA damage and illustrates the large number of proteins that are ubiquitinated during the DDR. [PubMed: 26051181]
27. Turnell AS, Grand RJ: DNA viruses and the cellular DNA-damage response. *J Gen Virol* 2012, 93:2076–2097. [PubMed: 22855786]
28. Lilley CE, Schwartz RA, Weitzman MD: Using or abusing: viruses and the cellular DNA damage response. *Trends in Microbiology* 2007, 15:119–126. [PubMed: 17275307]
29. Weitzman MD, Weitzman JB: What's the damage? The impact of pathogens on pathways that maintain host genome integrity. *Cell Host Microbe* 2014, 15:283–294. [PubMed: 24629335]
30. Mailand N, Bekker-Jensen S, Fastrup H, Melander F, Bartek J, Lukas C, Lukas J: RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. *Cell* 2007, 131:887–900. [PubMed: 18001824]
31. Huen MS, Grant R, Manke I, Minn K, Yu X, Yaffe MB, Chen J: RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. *Cell* 2007, 131:901–914. [PubMed: 18001825]
32. Kolas NK, Chapman JR, Nakada S, Ylanko J, Chahwan R, Sweeney FD, Panier S, Mendez M, Wildenhain J, Thomson TM, et al.: Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. *Science* 2007, 318:1637–1640.
33. Lilley CE, Chaurushiya MS, Boutell C, Landry S, Suh J, Panier S, Everett RD, Steward GS, Durocher D, Weitzman MD: A viral E3 ligase targets RNF8 and RNF168 to control histone ubiquitination and DNA damage responses. *Embo j* 2010, 29:943–955. [PubMed: 20075863]
34. Chaurushiya MS, Lilley CE, Aslanian A, Meisenhelder J, Scott DC, Landry S, Ticau S, Boutell C, Yates JR, 3rd, Schulman BA, et al.: Viral E3 ubiquitin ligase-mediated degradation of a cellular E3: viral mimicry of a cellular phosphorylation mark targets the RNF8 FHA domain. *Mol Cell* 2012, 46:79–90. [PubMed: 22405594]
35. Lees-Miller SP, Long MC, Kilvert MA, Lam V, Rice SA, Spencer CA: Attenuation of DNA-dependent protein kinase activity and its catalytic subunit by the herpes simplex virus type 1 transactivator ICP0. *J Virol* 1996, 70:7471–7477. [PubMed: 8892865]
36. Parkinson J, Lees-Miller SP, Everett RD: Herpes simplex virus type 1 immediate-early protein vmw110 induces the proteasome-dependent degradation of the catalytic subunit of DNA-dependent protein kinase. *J Virol* 1999, 73:650–657. [PubMed: 9847370]
37. Jackson SA, DeLuca NA: Relationship of herpes simplex virus genome configuration to productive and persistent infections. *Proc Natl Acad Sci U S A* 2003, 100:7871–7876. [PubMed: 12796511]
38. Giam CZ, Semmes OJ: HTLV-1 Infection and Adult T-Cell Leukemia/Lymphoma-A Tale of Two Proteins: Tax and HBZ. *Viruses* 2016, 8.
39. Petroski MD, Deshaies RJ: Function and regulation of cullin-RING ubiquitin ligases. *Nat Rev Mol Cell Biol* 2005, 6:9–20. [PubMed: 15688063]
40. Querido E, Blanchette P, Yan Q, Kamura T, Morrison M, Boivin D, Kaelin WG, Conaway RC, Conaway JW, Branton PE: Degradation of p53 by adenovirus E4orf6 and E1B55K proteins occurs via a novel mechanism involving a Cullin-containing complex. *Genes & Development* 2001, 15:3104–3117. [PubMed: 11731475]
41. Stracker TH, Carson CT, Weitzman MD: Adenovirus oncoproteins inactivate the Mre11-Rad50-NBS1 DNA repair complex. *Nature* 2002, 418:348–352.** This study demonstrated that the

- Adenovirus E1B455K/E4orf6 ligase targets the cellular DNA damage sensor MRN complex to prevent processing of the viral genome. [PubMed: 12124628]
42. Gupta A, Jha S, Engel DA, Ornelles DA, Dutta A: Tip60 degradation by adenovirus relieves transcriptional repression of viral transcriptional activator E1A. *Oncogene* 2013, 32:5017–5025. [PubMed: 23178490]
 43. Carson CT, Schwartz RA, Stracker TH, Lilley CE, Lee DV, Weitzman MD: The Mre11 complex is required for ATM activation and the G2/M checkpoint. *Embo j* 2003, 22:6610–6620. [PubMed: 14657032]
 44. Orazio NI, Naeger CM, Karlseder J, Weitzman MD: The adenovirus E1b55K/E4orf6 complex induces degradation of the Bloom helicase during infection. *J Virol* 2011, 85:1887–1892.
 45. Baker A, Rohleder KJ, Hanakahi LA, Ketner G: Adenovirus E4 34k and E1b 55k oncoproteins target host DNA ligase IV for proteasomal degradation. *J Virol* 2007, 81:7034–7040. [PubMed: 17459921]
 46. Blackford AN, Patel RN, Forrester NA, Theil K, Groitl P, Stewart GS, Taylor AM, Morgan IM, Dobner T, Grand RJ, et al.: Adenovirus 12 E4orf6 inhibits ATR activation by promoting TOPBP1 degradation. *Proc Natl Acad Sci U S A* 2010, 107:12251–12256. [PubMed: 20566845]
 47. Lakdawala SS, Schwartz RA, Ferencak K, Carson CT, McSharry BP, Wilkinson GW, Weitzman WD: Differential Requirements of the C Terminus of Nbs1 in Suppressing Adenovirus DNA Replication and Promoting Concatemer Formation. *J Virol* 2008, 82:8362–8372. [PubMed: 18562516]
 48. Shah GA, O'Shea CC: Viral and Cellular Genomes Activate Distinct DNA Damage Responses. *Cell* 2015, 162:987–1002. [PubMed: 26317467]
 49. Evans JD, Hearing P: Relocalization of the Mre11-Rad50-Nbs1 complex by the adenovirus E4 ORF3 protein is required for viral replication. *J Virol* 2005, 79:6207–6215. [PubMed: 15858005]
 50. Karen KA, Hoey PJ, Young CS, Hearing P: Temporal regulation of the Mre11-Rad50-Nbs1 complex during adenovirus infection. *J Virol* 2009, 83:4565–4573. [PubMed: 19244322]
 51. Zhao X, Madden-Fuentes RJ, Lou BX, Pipas JM, Gerhardt J, Rigell CJ, Fanning E: Ataxia telangiectasia-mutated damage-signaling kinase-and proteasome-dependent destruction of Mre11-Rad50-Nbs1 subunits in Simian virus 40-infected primate cells. *J Virol* 2008, 82:5316–5328. [PubMed: 18353955]
 52. Levine AJ: The common mechanisms of transformation by the small DNA tumor viruses: The inactivation of tumor suppressor gene products: p53. *Virology* 2009, 384:285–293. [PubMed: 19081592]
 53. Cai QL, Knight JS, Verma SC, Zald P, Robertson ES: EC5S ubiquitin complex is recruited by KSHV latent antigen LANA for degradation of the VHL and p53 tumor suppressors. *PLoS Pathog* 2006, 2:e116. [PubMed: 17069461]
 54. Gonzalez SL, Stremlau M, He X, Basile JR, Munger K: Degradation of the retinoblastoma tumor suppressor by the human papillomavirus type 16 E7 oncoprotein is important for functional inactivation and is separable from proteasomal degradation of E7. *J Virol* 2001, 75:7583–7591. [PubMed: 11462030]
 55. Huh K, Zhou X, Hayakawa H, Cho JY, Libermann TA, Jin J, Harper JW, Munger K: Human papillomavirus type 16 E7 oncoprotein associates with the cullin 2 ubiquitin ligase complex, which contributes to degradation of the retinoblastoma tumor suppressor. *J Virol* 2007, 81:9737–9747. [PubMed: 17609271]
 56. Sato Y, Kamura T, Shirata N, Murata T, Kudoh A, Iwahori S, Nakayama S, Isomura H, Nishiyama Y, Tsurumi T: Degradation of phosphorylated p53 by viral protein-ECS E3 ligase complex. *PLoS Pathog* 2009, 5:e1000530. [PubMed: 19649319]
 57. Sato Y, Shirata N, Kudoh A, Iwahori S, Nakayama S, Murata T, Isomura H, Nishiyama Y, Tsurumi T: Expression of Epstein-Barr virus BZLF1 immediate-early protein induces p53 degradation independent of MDM2, leading to repression of p53-mediated transcription. *Virology* 2009, 388:204–211. [PubMed: 19375142]
 58. Knight JS, Sharma N, Robertson ES: Epstein-Barr virus latent antigen 3C can mediate the degradation of the retinoblastoma protein through an SCF cellular ubiquitin ligase. *Proc Natl Acad Sci U S A* 2005, 102:18562–18566. [PubMed: 16352731]

59. Li T, Robert EI, van Breugel PC, Strubin M, Zheng N: A promiscuous alpha-helical motif anchors viral hijackers and substrate receptors to the CUL4-DDB1 ubiquitin ligase machinery. *Nat Struct Mol Biol* 2010, 17:105–111. [PubMed: 19966799]
60. Murphy CM, Xu Y, Li F, Nio K, Reszka-Blanco N, Li X, Wu Y, Yu Y, Xiong Y, Su L: Hepatitis B Virus X Protein Promotes Degradation of SMC5/6 to Enhance HBV Replication. *Cell Rep* 2016, 16:2846–2854.** This study, along with Decorsiere et. al., shows that HBV protein HBx integrates into a cellular cullin complex to ubiquitinate and degrade SMC5/6. SMC5/6 is identified as a host restriction factor. [PubMed: 27626656]
61. Decorsiere A, Mueller H, van Breugel PC, Abdul F, Gerossier L, Beran RK, Livingston CM, Niu C, Fletcher SP, Hantz O, et al.: Hepatitis B virus X protein identifies the Smc5/6 complex as a host restriction factor. *Nature* 2016, 531:386–389.** This study, along with Murphy et. al., shows that HBV protein HBx integrates into a cellular cullin complex to ubiquitinate and degrade SMC5/6. SMC5/6 is identified as a host restriction factor. [PubMed: 26983541]
62. Scheffner M, Huibregtse JM, Vierstra RD, Howley PM: The HPV-16 E6 and E6-AP Complex Functions as a Ubiquitin-Protein Ligase in the Ubiquitination of p53. *Cell* 1993, 75:495–505.** This landmark study demonstrated how the viral E6 oncoprotein of HPV harnesses ubiquitination to target p53 for proteasomal degradation. [PubMed: 8221889]
63. Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM: The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell* 1990, 63:1129–1136. [PubMed: 2175676]
64. Huibregtse JM, Scheffner M, Howley PM: A cellular protein mediates association of p53 with the E6 oncoprotein of human papillomavirus types 16 or 18. *Embo j* 1991, 10:4129–4135. [PubMed: 1661671]
65. Subbiah VK, Zhang Y, Rajagopalan D, Abdullah LN, Yeo-Teh NS, Tomaic V, Banks L, Myers MP, Chow EK, Jha S: E3 ligase EDD1/UBR5 is utilized by the HPV E6 oncogene to destabilize tumor suppressor TIP60. *Oncogene* 2016, 35:2062–2074.* This study identifies a cellular E3 ligase, EDD1, interacting with a DDR protein, TIP60, and modulation of it's effect by a virus causing indirect ubiquitin-related impact on TIP60. [PubMed: 26234678]
66. White EA, Munger K, Howley PM: High-Risk Human Papillomavirus E7 Proteins Target PTPN14 for Degradation. *MBio* 2016, 7.
67. Munakata T, Liang Y, Kim S, McGivern DR, Huibregtse J, Nomoto A, Lemon SM: Hepatitis C virus induces E6AP-dependent degradation of the retinoblastoma protein. *PLoS Pathog* 2007, 3:1335–1347. [PubMed: 17907805]
68. Li L, Li W, Xiao L, Xu J, Chen X, Tang M, Dong Z, Tao Q, Cao Y: Viral oncoprotein LMP1 disrupts p53-induced cell cycle arrest and apoptosis through modulating K63-linked ubiquitination of p53. *Cell Cycle* 2012, 11:2327–2336. [PubMed: 22684299]
69. Kwun HJ, Shuda M, Feng H, Camacho CJ, Moore PS, Chang Y: Merkel cell polyomavirus small T antigen controls viral replication and oncoprotein expression by targeting the cellular ubiquitin ligase SCFFbw7. *Cell Host Microbe* 2013, 14:125–135. [PubMed: 23954152]
70. Kwun HJ, Wendzicki JA, Shuda Y, Moore PS, Chang Y: Merkel cell polyomavirus small T antigen induces genome instability by E3 ubiquitin ligase targeting. *Oncogene* 2017, 36:6784–6792. [PubMed: 28846109]
71. Whitehurst CB, Vaziri C, Shackelford J, Pagano JS: Epstein-Barr virus BPLF1 deubiquitinates PCNA and attenuates polymerase eta recruitment to DNA damage sites. *J Virol* 2012, 86:8097–8106. [PubMed: 22623772]
72. Gastaldello S, Hildebrand S, Faridani O, Callegari S, Palmkvist M, Di Guglielmo C, Masucci MG: A deneddylase encoded by Epstein-Barr virus promotes viral DNA replication by regulating the activity of cullin-RING ligases. *Nat Cell Biol* 2010, 12:351–361. [PubMed: 20190741]
73. Everett RD, Meredith M, Orr A, Cross A, Kathoria M, Parkinson J: A novel ubiquitin-specific protease is dynamically associated with the PML nuclear domain and binds to a herpesvirus regulatory protein. *Embo j* 1997, 16:1519–1530. [PubMed: 9130697]
74. Kim RQ, Sixma TK: Regulation of USP7: A High Incidence of E3 Complexes. *J Mol Biol* 2017, 429:3395–3408. [PubMed: 28591556]

75. Smits VA, Freire R: USP7/HAUSP: A SUMO deubiquitinase at the heart of DNA replication. *Bioessays* 2016, 38:863–868. [PubMed: 27374980]
76. Li M, Chen D, Shiloh A, Luo J, Nikolaev AY, Qin J, Gu W: Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization. *Nature* 2002, 416:648–653. [PubMed: 11923872]
77. Li M, Brooks CL, Kon N, Gu W: A dynamic role of HAUSP in the p53-Mdm2 pathway. *Mol Cell* 2004, 13:879–886. [PubMed: 15053880]
78. Zhu Q, Sharma N, He J, Wani G, Wani AA: USP7 deubiquitinase promotes ubiquitin-dependent DNA damage signaling by stabilizing RNF168. *Cell Cycle* 2015, 14:1413–1425. [PubMed: 25894431]
79. An L, Jiang Y, Ng HH, Man EP, Chen J, Khoo US, Gong Q, Huen MS: Dual-utility NLS drives RNF169-dependent DNA damage responses. *Proc Natl Acad Sci U S A* 2017, 114:E2872–e2881. [PubMed: 28325877]
80. Holowaty MN, Zeghouf M, Wu H, Tellam J, Athanasopoulos V, Greenblatt J, Frappier L: Protein profiling with Epstein-Barr nuclear antigen-1 reveals an interaction with the herpesvirus-associated ubiquitin-specific protease HAUSP/USP7. *J Biol Chem* 2003, 278:29987–29994. [PubMed: 12783858]
81. Jager W, Santag S, Weidner-Glunde M, Gellermann E, Kati S, Pietrek M, Viejo-Borbolla A, Schulz TF: The ubiquitin-specific protease USP7 modulates the replication of Kaposi's sarcoma-associated herpesvirus latent episomal DNA. *J Virol* 2012, 86:6745–6757. [PubMed: 22514345]
82. Ching W, Koyuncu E, Singh S, Arbelo-Roman C, Hartl B, Kremmer E, Speiseder T, Meier C, Dobner T: A ubiquitin-specific protease possesses a decisive role for adenovirus replication and oncogene-mediated transformation. *PLoS Pathog* 2013, 9:e1003273. [PubMed: 23555268]
83. Sheng Y, Saridakis V, Sarkari F, Duan S, Wu T, Arrowsmith CH, Frappier L: Molecular recognition of p53 and MDM2 by USP7/HAUSP. *Nat Struct Mol Biol* 2006, 13:285–291. [PubMed: 16474402]
84. Saridakis V, Sheng Y, Sarkari F, Holowaty MN, Shire K, Nguyen T, Zhang RG, Liao J, Lee W, Edwards AM, et al.: Structure of the p53 binding domain of HAUSP/USP7 bound to Epstein-Barr nuclear antigen 1 implications for EBV-mediated immortalization. *Mol Cell* 2005, 18:25–36. [PubMed: 15808506]
85. Liu X, Li Y, Peng S, Yu X, Li W, Shi F, Luo X, Tang M, Tan Z, Bode AM, et al.: Epstein-Barr virus encoded latent membrane protein 1 suppresses necroptosis through targeting RIPK1/3 ubiquitination. *Cell Death Dis* 2018, 9:53. [PubMed: 29352166]
86. Duncan LM, Piper S, Dodd RB, Saville MK, Sanderson CM, Luzio JP, Lehner PJ: Lysine-63-linked ubiquitination is required for endolysosomal degradation of class I molecules. *The EMBO journal* 2006, 25:1635–1645.
87. Zhang Q, Karnak D, Tan M, Lawrence TS, Morgan MA, Sun Y: FBXW7 Facilitates Nonhomologous End-Joining via K63-Linked Polyubiquitylation of XRCC4. *Mol Cell* 2016, 61:419–433. ** This study shows a novel mechanism of CRL mediating non-degradative K63-polyubiquitin within the DDR. [PubMed: 26774286]
88. Lee SW, Li CF, Jin G, Cai Z, Han F, Chan CH, Yang WL, Li BK, Rezaeian AH, Li HY, et al.: Skp2-dependent ubiquitination and activation of LKB1 is essential for cancer cell survival under energy stress. *Mol Cell* 2015, 57:1022–1033. * This study shows non-degradative ubiquitination induced by a CRL. [PubMed: 25728766]
89. Xu G, Paige JS, Jaffrey SR: Global analysis of lysine ubiquitination by ubiquitin remnant immunoaffinity profiling. *Nat Biotechnol* 2010, 28:868–873. [PubMed: 20639865]
90. Udeshi ND, Mertins P, Svinkina T, Carr SA: Large-scale identification of ubiquitination sites by mass spectrometry. *Nat Protoc* 2013, 8:1950–1960.

Figure 1: Viral strategies to hijack the cellular ubiquitin system.

Viruses encode proteins with E3 ubiquitin activity that directly ubiquitinate cellular substrates (I). Viral proteins integrate into cellular cullin-RING E3 ligases, assuming the substrate adaptor function to dictate cellular substrates that are ubiquitinated (II). Viral proteins interact with cellular E3 ligases to direct ubiquitination of cellular substrates or alter cellular E3 ligase activity (III). Viruses can impart the opposite function of E3 ligases by directing deubiquitination of cellular substrates either by encoding viral proteins with DUB activity or by interacting with cellular DUBs (IV). Studying the repertoire of mechanisms that viruses use to exploit the host ubiquitin system will broaden our understanding of host-pathogen interactions and functions of ubiquitin in cellular processes such as the DDR.

Figure 2: Viruses target DDR pathways using multiple ubiquitination strategies.

Viruses use ubiquitination to target the PIKK and PCNA DDR pathways at the levels of the “sensors”, “mediators” and “effectors” of DDR signaling. Viruses also target downstream responses of the DDR, especially by degradation of tumor suppressors such as p53, which regulates cell cycle checkpoints and apoptosis. Each of the mechanisms of viral-mediated ubiquitination (Figure 1) can be identified in viral manipulation of DDR signaling.