

Extracellular events impacting human papillomavirus infections: Epithelial wounding to cell signaling involved in virus entry



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ABSTRACT

Human papillomaviruses (HPVs), like all PVs, predominantly cause benign tumors, or warts, in stratifying squamous epithelial tissues. Virions are released from apical surfaces of the skin and mucosa and, to initiate a new infection, must utilize a break in the epithelial barrier to access mitotically active basal epithelial cells. Laboratory models currently used to study the HPV infectious process reveal that heparan sulfate proteoglycans and cellular enzymes are utilized to prime virions and activate cell signaling to coordinate virus association with a receptor complex for uptake into keratinocytes. Conventional cell-based infection systems lack many aspects relevant to determining the role of epithelial wounding in HPV infections. Nevertheless, many cellular factors involved in virion interaction with cells have been shown to actively coordinate their activities in the dynamic state of an epithelial wound. In this review, I summarize the current knowledge regarding how HPVs interact with extracellular components to prime virus particles for eventual disassembly and effectuate association with the viral receptor complex. Additionally, I propose a model to account for how epithelial injury and the wound response may actively participate in successful HPV infection of basal epithelial cells.

1. The epithelial tissue context of HPV infection

Papillomaviruses (PVs) initiate productive infections in stratifying and differentiating epithelium, which is the first line of defense against invading microorganisms. Wounding or micro-abrasion of the epithelium has been recognized for nearly 100 years to be important in facilitating efficient PV infections *in vivo* [1]. The most obvious explanation is that a break in the protective cell layers allows virions to access the mitotically-competent basal epithelial cells able to support the establishment of episomal viral genome replication. Yet, the wound state is dynamic and likely provides soluble factors and cellular changes that are usurped by PVs for efficient infection *in vivo*. The lack of tractable and physiologically replete tissue-based models *in vitro* has hampered our ability to fully assess the role that epithelial injury plays in early PV infection events. Here, I aim to summarize the current understanding of how oncogenic human PVs (HPVs) interact with their natural host cell, the squamous epithelial cell. I will highlight the roles of the identified host cell attachment factors, enzymes and bioactive molecules in priming the HPV capsid prior to cellular uptake, the latter

of which is reviewed by Mikuličić and Florin in this issue. The importance of cell surface signaling events necessary for the assembly of the viral entry receptor complex will be discussed. Critical gaps in knowledge also will be noted. Being mindful that successful infection *in vivo* requires a wounded epithelium, many roles these cellular factors play in epithelial wounding are summarized in Table 1.

Infections by the oncogenic HPV genotypes 16, 18, 31, 33 and the low-risk HPV11 are the best studied HPVs at the cellular and molecular levels. Due to the difficulties associated with isolating high-titer, oncogenic HPV virions from differentiating epithelial tissues, most studies on the initial stages of HPV infection have relied on surrogate particles. Ectopic expression and self-assembly of the L1 and L2 capsid proteins in simple cell cultures facilitates the production of virus-like particles, pseudovirions (PsV) and quasivirions (QV). PsVs comprise viral particles packaging a pseudogenome, which encodes a reporter protein used as a readout of successful genome nuclear delivery [2]. Similarly, QVs encapsidate the HPV circular, double-stranded DNA genome, including viral genomes engineered with genetic modifications [3]. Subsequent to successful virion entry and trafficking, viral (or pseudoviral) gene

Abbreviations: A2t, the annexin A2/S100A10 heterotetramer; ADAM, a disintegrin and metalloproteinase; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; GF, growth factor; HPV, human papillomavirus; FAK, focal adhesion kinase; GAG, glycosaminoglycan; HS, heparan sulfate; MMP, matrix metalloproteinase; PI3K, phosphatidylinositol 3-kinase; RTK, receptor tyrosine kinase; Sdc, syndecan

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Table 1
Wound-related expression and activities of HPV interacting partners and molecules critical for efficient infection.

Molecules	Activities	References
ADAM sheddases (a disintegrin and metalloproteinase)	Increases the levels of HS-containing Sdc ectodomains in wound fluids to augment healing Process GF transmembrane precursors to soluble forms	Bertrand et al. (2019) <i>Br. J. Pharmacol.</i> , 176:67.; Manon-Jensen et al. (2010) <i>FEBS J</i> , 277:3876. Martins et al. (2013) <i>Cell Tissue Res.</i> , 351:255.
Annexin A2 (AnxA2)	Expression is upregulated at the epithelial wound edge Regulates EGFR endocytosis and signaling Regulates actin-associated cellular processes at dynamic membranes; regulates cargo trafficking	Stoscheck et al. (1992) <i>J. Invest. Dermatol.</i> , 99:645, Munz et al. (1997) <i>J. Invest. Dermatol.</i> , 108:307. Grewal et al. (2009) <i>Cell. Signal.</i> , 21:847. Grieve et al. (2012) <i>Int. J. Cell Biol.</i> , 2012:852430.
Matrix Metallo-proteinases (MMPs)	Increase the levels of HS-containing Sdc ectodomains in wound fluids to augment healing Upregulated by GFR and integrin signaling at the wound edge; Process GF transmembrane precursors to soluble forms	Bertrand (2019) <i>Br. J. Pharmacol.</i> , 176:67.; Manon-Jensen (2010) <i>FEBS J</i> , 277:3876. Martins (2013) <i>Cell Tissue Res.</i> , 351:255.
Epidermal growth factor receptor (EGFR)	Expression is upregulated at the epithelial wound edge	Stoscheck (1992) <i>J. Invest. Dermatol.</i> , 99:645, Munz (1997) <i>J. Invest. Dermatol.</i> , 108:307.
Furin	Activates proteins vital to proper cellular functioning and wound response: GFs and hormones, receptors, MMPs and ADAM sheddases	Seidah et al. (2012) <i>Nat. Rev. Drug Disc.</i> , 11:367.
Heparinase	Liberates GF-bound HS polymers from HSPGs and the ECM to enhance wound repair Regulates syndecan clustering and signaling; induces EGFR and Src signaling	Nasser (2008) <i>Cell. Molec. Life Sci.</i> , 65:1706. Fux et al. (2009) <i>Trends Biochem Sci.</i> 34:511.
$\alpha 6\beta 4$ Integrin	Expression limited to basal epithelium in hemidesmosomes; interacts with LN332 in ECM Expression is upregulated in a wound	Longmate et al. (2014) <i>Adv. Wound Care</i> , 3:229. Longmate et al. (2014) <i>Adv. Wound Care</i> , 3:229.
Kallikrein-related peptidase 8 (KLK8)	Roles in ECM remodeling and wound closure Roles in desquamation of cornified cells	Kishibe et al. (2012) <i>J. Invest. Dermatol.</i> , 132:1717. Kishibe et al. (2007) <i>J. Biol. Chem.</i> , 282:5834.
Keratinocyte growth factor receptor (KGFR), also denoted FGFR2IIIb	Expression is upregulated at the epithelial wound edge	Stoscheck (1992) <i>J. Invest. Dermatol.</i> , 99:645, Munz (1997) <i>J. Invest. Dermatol.</i> , 108:307.
Syndecan-1 (Sdc1)	Expression increases at the epithelial wound edge; regulates cell adhesion, cell migration, and endocytosis Clustering with Her1 GFR and $\alpha 6\beta 4$ integrin promotes wound healing	Bertrand (2019) <i>Br. J. Pharmacol.</i> , 176:67, Stepp et al. (2015) <i>Adv. Wound Care</i> , 4:235. Afratis et al. (2017) <i>FEBS J.</i> , 284:27.
Sdc4	Expression increases at the epithelial wound edge Clustering with EGFR and $\alpha 6\beta 4$ integrin promotes wound healing	Gallo et al. (1996) <i>J. Invest. Dermatol.</i> , 107:676. Afratis (2017) <i>FEBS J.</i> , 284:27.
Tetraspanins	Regulators of EGFR signaling and trafficking; influence integrin signaling via their interactions with FAK Regulate expression of MMPs and ADAMs	Termini et al. (2017) <i>Front. Cell Dev. Biol.</i> , 5:34. Jiang et al. (2015) <i>Cell Adh. Migr.</i> , 9:406.
Tetraspanin CD151	Upregulated in wounded epidermis; interacts with $\alpha 6\beta 4$ integrin and FAK	Longmate (2014) <i>Adv. Wound Care</i> , 3:229.

expression is quantified as a measure of successful infection. The specifics of HPV virion source is not known to impact interaction with host cells. However, virions spread in nature are released in the context of desquamating cornified cells (DCC) from the apical surface of lesions and are not transmitted in purified form. In contrast, most studies to be described below used HPV particles isolated from whole-cell or whole-tissue lysates, and typically after density gradient centrifugation. Although varied degrees of virus purification are central methods in virology, they may remove soluble or virion-associated factors that may be crucial for transmission *in vivo*.

2. Heparan sulfate proteoglycan (HSPG) interactions

Many viruses and intracellular pathogens that infect anogenital mucosa initially interact with their target host cells via the glycosaminoglycan (GAG) chains of HSPGs (reviewed in Ref. [4]). HSPGs are ubiquitously expressed on the cell surface of adherent cells and are present in the cell-secreted extracellular matrix (ECM). HSPGs are composed of one or several heparan sulfate (HS) GAG chains covalently

attached to the ectodomains of specific core proteins [5]. For most viruses, interaction with HSPGs is considered an initial, non-specific contact that facilitates capsid conformational changes allowing virion transfer to secondary high-affinity, virus-specific entry receptor(s). However, accumulating evidence suggests that HSPGs could have more active roles in the infectious uptake of pathogens, particularly of HPVs (see Fig. 1).

HPV particles attach to the HSPGs syndecan 1 (Sdc1) and Sdc4 on keratinocytes [6], (Fig. 1Biii) and expression of both is increased at the epithelial wound edge (see Table 1). Sdcs link the cytoskeleton to the ECM: the HS-linked ectodomains of Sdcs interact with ECM components, whereas Sdc cytoplasmic domains interact with kinases and the cytoskeleton. Sdcs act as co-receptors for many growth factors (GFs) and their cognate GF receptors (GFRs) to initiate cell signaling. Sdc1 and Sdc4 associate with GFRs and $\alpha 6\beta 4$ integrin to promote wound healing. Notably, HPVs interact with epidermal GFR (EGFR), keratinocyte GFR (KGFR) and $\alpha 6\beta 4$ integrin, and signaling from these molecules is important for efficient HPV infection (described below in section 4).

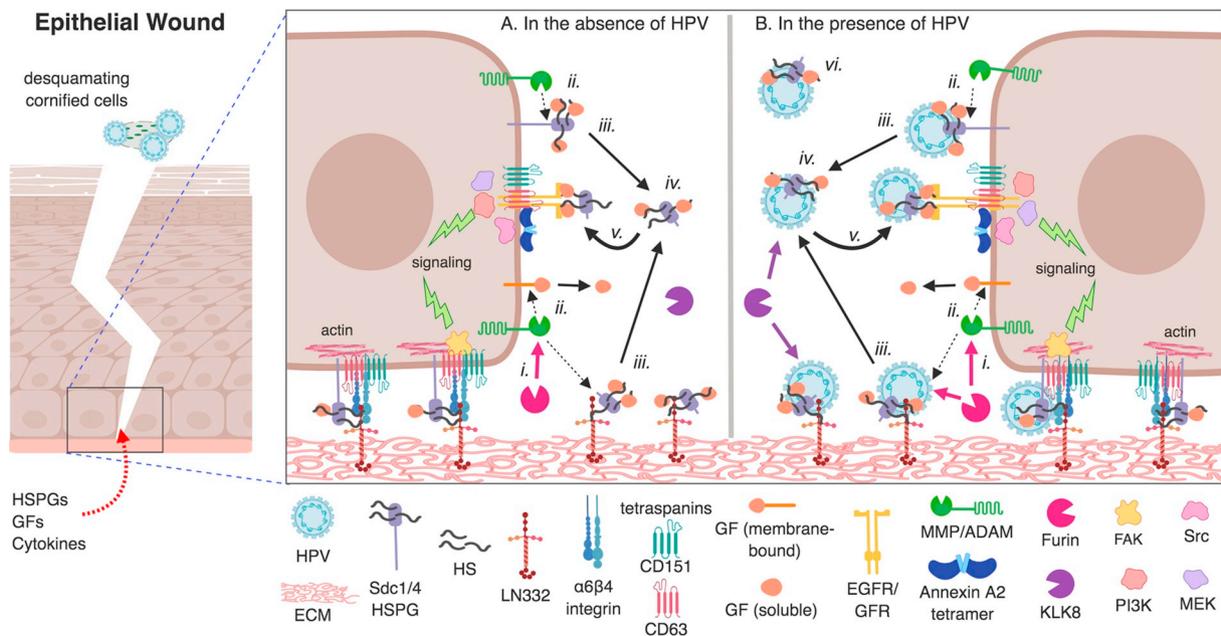


Fig. 1. Proposed model for HPV extracellular interactions in a dynamic wounded microenvironment where virions are associated with DCCs. **(A).** Natural processes that occur in the absence of HPV. The basal edges of epithelial cells contact the ECM consisting of collagens, elastins, fibronectins, and laminins. LN332 interacts with Sdc1, CD151 tetraspanin and $\alpha 6 \beta 4$ integrin on the basal cell to provide cell anchorage to the ECM/basement membrane, termed the hemidesmosome. (i.) Proprotein convertases, like furin, activate MMPs and ADAM sheddases (ii), which catalyze the release or “shedding” of membrane-bound GFs and the protein ectodomains of HSPG, including Sdc1 and Sdc4 (dotted arrows). (iii.) HSPG in the plasma membrane and ECM act as local depots for soluble GFs and other bioactive molecules. (iv.) Soluble complexes containing GFs and HSPGs are liberated by heparinases and proteolytic processing of LN332. (v.) Soluble GF complexes bind to GFRs and activate intracellular signaling cascades. EGFR-mediated Src signaling activates the A2t to transport to the plasma membrane surface. A2t and CD151 regulate EGFR endocytosis. **(B).** When present, HPVs hijack the normal processes of HSPG decoration with GFs and their release from the cells. By virtue of HPV particle interaction with HS, KLK8 cleaves L1, furin processes L2 and promotes sheddase-mediated release of HSPG- and GF-bound HPV. These functions foster HPV decoration with HS and GFs (iv) and signaling, leading to virus interaction with the receptor complex (v). (vi.) HPV virions may associate with soluble HS-GF complexes in the wound and *in vivo* may also arrive in the wound *milieu* with the ability to induce signaling to mobilize the receptor complex. Image created with BioRender.

Under physiological conditions, the HS-containing ectodomains of Sdcs are constitutively cleaved by a variety of sheddases, a process termed “ectodomain shedding” (Fig. 1Aii). Sdc sheddases include matrix metalloproteinases (MMPs) and ADAMs (a disintegrin and metalloproteinases), which increase the levels of HS-containing Sdc ectodomains in wound fluids. MMPs and ADAMs also process GF transmembrane precursors, releasing soluble GFs. Additionally, heparinase liberates GF-bound HS polymers from HSPGs. Processed HS molecules are more bioactive than their native HS chains, and act as potent promoters of GF activity. Work in my laboratory demonstrated that HPV infection strongly depends on MMPs, ADAM sheddases, and heparinases to release HS and Sdc-1 ectodomains [7,8]. We found keratinocyte- and ECM-bound HPV particles subsequently released by MMPs remain associated with HS, HSPGs and EGF-family GFs, an observation we termed as “HPV particle decoration” (Fig. 1Biii–iv). These soluble decorated HPV virions were able to infect cells deficient in HSPGs, supporting the notion that HS-bound virions can bypass the need for cellular HSPGs [7].

Positively-charged L1 motifs in HPV capsids mediate ionic interaction with the negatively-charged HS molecules on HSPGs [9,10]. Numerous studies using L1-only VLPs, PsVs and HPV11 virions derived from the mouse xenograft system show that HPV particles preferentially attach to HSPGs present in the ECM present beneath and adjacent to keratinocytes in cell culture and in the basement membrane of epithelial tissues. These observations were made using monolayer keratinocyte cultures, cervical tissue sections and the mouse vaginal challenge model [9,11,12]. Laminin-332 (LN332; formerly, “LN5”), an essential component of the dermal-epidermal basement membrane, is an ECM-resident receptor for HSPGs and the $\alpha 6 \beta 4$ integrin. LN332 in the ECM of cultured cells also harbors a significant mass of processed HS and shed HSPG ectodomains. Thus, it is not surprising that HPV

particles also localize with LN332. The Sapp laboratory found that HPV capsids can also interact directly with LN332 [13].

HPV particles also interact substantially with HSPGs on the plasma membrane of keratinocytes [6,11,13–16]. HPV particles bind cells with high affinity [17], an interaction that is consistent with the high-affinity HPV capsid binding to heparin, a GAG polysaccharide resembling heparan sulfate. Joyce et al. showed that the HPV-heparin interaction affinity was similar to that of other heparin-binding proteins [9].

Removal of HSPGs from keratinocyte cultures using heparinase or chlorate treatment, which prevents sulfonation of GAGs, demonstrated that these molecules are required for efficient infection with HPV16 and HPV33 PsVs and xenograft-derived HPV11 [11,14]. Studies using cells defective for GAG biosynthesis and heparinase treatment of the mouse vaginal tract corroborate that HSPGs are required for robust PsV infections [12,18]. My laboratory reported that cell-associated HSPGs were dispensable for HPV infections using organotypic (raft) epithelial tissue-derived HPV31 virion infection of keratinocytes [19]. Likewise, Day et al. concluded that cell-associated HSPGs were not required for infection by HPV16 PsVs treated with conditioned medium from cells overexpressing furin, an enzyme important for L2 processing (discussed further below) [18]. Regrettably, neither of these studies considered that HSPGs, or indeed other factors, might be present in the virus inocula. Recently, my group found that HS molecules present with raft tissue-derived virions and in furin-conditioned medium can rescue the requirement for cell-associated HSPGs during infection (ms. in preparation). This agrees with the finding that heparin-preincubated virions and HS-GF-decorated virions also bypassed the requirement for cell-associated HSPGs [7,20]. Thus, the initial conclusions that HSPGs were not involved in raft-derived HPV31 or furin-mediated HPV16 PsV infections were naïve, and indeed, not fully tested by the experimental approaches. Together, these findings call into question the concept of a

heparan sulfate independent infection route for HPVs. Whether HPV virions liberated in DCCs from lesions *in vivo* are associated with HSPG or other factors that help promote infection has yet to be investigated.

3. Capsid priming prior to endocytosis

HPV capsids are highly stable structures responsible for protecting the viral genome and facilitating its delivery to naïve host cells [21]. HPV capsid interactions with HSPG are largely thought to provide a platform for HPV capsid conformational shifts and enzymatic processing steps that are important for proper L2-mediated, post-entry trafficking of the viral genome to the nucleus. Many studies infer that HSPG binding leads to conformational changes in L1, the major capsid protein. The indirect evidence includes the appearance of a neutralizing epitope at the N-terminus of the L2 minor capsid protein (denoted as the “RG-1” epitope between aa 17–36) and the accessibility of L1 epitope(s), which appear predominantly unreactive in mature PsVs. Incubation of PsVs with soluble heparin facilitates L1 cleavage by the secreted trypsin-like serine protease kallikrein-8 (KLK8), which results in accessibility of the L2 RG-1 epitope [20].

The role of furin, a proprotein convertase, in HPV L2 processing during infection has been widely studied. A highly-conserved cleavage site for furin is situated a few amino acids upstream of the RG-1 epitope in L2 [22]. The Sapp group initially showed that cyclophilin B activity is important for furin's accessibility to the N-terminus of L2 at the cell surface; this was reproduced by the Schelhaas team [20,23]. Although the Campos lab found that furin cleaves an N-terminally tagged L2 in the presence of cyclophilin inhibitors, the tag may alter L2's conformation in the virion alleviating the need for cyclophilin [24]. Furin cleavage of L2 may occur at the plasma membrane; however, furin inhibition is not manifest until the endosome where escape of the L2/vDNA complex is inhibited [22].

After capsid priming at the cell surface or on the ECM, these conformational changes are suggested to cause HPV particles to dissociate from high-affinity HS and HSPG interactions and to move to an entry receptor complex that is subsequently independent of HS activities. However, as noted above, our studies indicate that infection is dependent upon the actions of sheddases and heparinases to release HS-decorated viral capsids from the ECM and plasma membrane (Fig. 1Biii) [7,8]. Although furin was proposed to regulate extracellular HPV-keratinocyte interactions or transfer of ECM-attached virions to receptors responsible for virus entry, we found no evidence that furin is involved in these processes [8]. Whether HS molecules remain associated with HPV virions during entry and trafficking has not been reported.

Other potential roles for furin in HPV infectious uptake have yet to be fully explored. Particularly, furin and other proprotein convertases activate GFs, hormones, receptors, MMPs and ADAM sheddases (see Table 1). Consistent with these activities of furin, as noted above we find that conditioned medium from cells overexpressing furin contains high levels of HS, which supports HPV infection of HSPG-null cells in an HS-dependent manner. Furin-induced sheddase processing of GFs and receptors may also contribute to HPV infection (Fig. 1Bi).

4. Cell signaling required for initiating HPV infection

Signaling related to epithelial wound responses is involved in HPV infections. HPV-induced signaling promotes actin reorganization and activation of filopodia for virion acquisition from the ECM [25]. FAK signaling via $\alpha 6\beta 4$ integrin and signaling through EGFR and KGFR are each important for HPV infection [7,26,27]. Remarkably, tetraspanins CD63 and CD151, which are involved in HPV entry (see Mikuličić and Florin in this issue), influence integrin signaling via their interactions with FAK; tetraspanins are also regulators of EGFR signaling and trafficking. HPV-activated EGFR signaling initiates the downstream activation of PI3K to block autophagy, an intrinsic sensor that inhibits efficient HPV infectious entry [16]. EGFR signaling also activates Src

kinase to phosphorylate annexin A2. This promotes the extracellular translocation of the annexin A2/S100A10 heterotetramer (A2t) [28], a complex that interacts with EGFR and regulates its uptake into cells (Table 1).

5. Conclusions and critical gaps in knowledge

A model is emerging as to how HPVs usurp normal cellular activities to promote efficient infection into squamous epithelial cells (Fig. 1). However, neither the role of epithelial wounding, nor the virion-associated factors from DCCs are fully appreciated during the process of HPV infections. *In vivo* wounds demonstrate an influx of serum containing HSPGs, GFs, and cytokines to promote wound healing. During this process, the integrin adhesions of basal keratinocytes to the ECM/basement membrane are dissolved allowing cell migration through the wound matrix. MMPs, ADAMs, and heparinases cleave HSPGs and HS chains, yielding soluble, HS-bound GFs that are high-affinity ligands of their cognate receptors. ECM proteins including LN322 are deposited and accumulate HSPG, HS polymers, and GFs, and filopodia are induced to promote HK migration to close the wound. The expression of many molecules involved in HPV infection is upregulated during wounding (Table 1). The spatio-temporal regulation of these processes is likely an important factor in how HPVs interact and are primed for association with the entry complex prior to infectious uptake. Future work must strive to incorporate aspects of virion transmission in the context of DCCs and the wound response to better define the molecular components and cellular processes required for HPV interactions in this dynamic state.

Competing interests

The author declares that this review was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.pvr.2019.04.009>.

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